ANNUAL REPORT

Division of Intramural Research Programs
National Institute of Mental Health

October 1, 1984 - September 30, 1985

VOLUME II PART I
INDIVIDUAL PROJECT REPORTS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health
Division of Intramural Research Programs

ANNUAL REPORT

DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH (U.S.)

October 1, 1984 - September 30, 1985

VOLUME II PART I

INDIVIDUAL PROJECT REPORTS

ANNUAL REPORT
DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

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DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

RESEARCH PROJECT SERIAL NUMBER LISTING:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00092-11 BP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Central Amines and Aggression, Suicide, and Alcoholism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gerald L. Brown, M.D,, Medical Officer, BPB, NIMH

Dr. Frederick K. Goodwin, Scientific Director, IRP, NIMH; Dr. Robert M. Post, Chief, Biological Psychiatry Branch, NIMH; Dr. Markku Linnoila, Chief, Lab. of Clinical Studies, NIAAA; Cmdr. Peter F. Goyer, M.D., Dept. of Psychiatry, Portsmouth Naval Medical Center; Captain O.L. Royal, M.C., USN, National Medical Center; Dr. Richard J. Wyatt, St. Elizabeth's Hospital, NIMH

COOPERATING UNITS *(if any)*

Intramural Research Program, NIMH
Laboratory of Clinical Studies, NIAAA; Department of Psychiatry
National Naval Medical Center; Portsmouth Naval Medical Center

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.70	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The National Institute of Mental Health (NIMH), both separately and together with the National Naval Medical Center, is studying central amine metabolites in the cerebrospinal fluid (CSF) of psychiatric patients. Results to date indicate that aggression and anti-social behavior are inversely correlated with CSF 5-hydroxy-indoleacetic acid (5HIAA). Low CSF 5HIAAA is also associated with suicidal history; suicidal history is similarly associated with a history of aggressive, anti-social behavior. Thus, a trivariate relationship exists among history of aggression, history of suicidal behavior, and lower CSF 5HIAA. CSF 5HIAA is inversely related to the Pd scale. Findings have been largely replicated in two separate populations. Alcoholics have decreased CSF 5HIAA during abstinence. Disulfiram (Antabuse) appears to lower CSF homovanillic acid (HVA) and appears to increase serum norepinephrine (NE); low CSF dopamine-beta-hydroxylase (DBH), low platelet monoamine oxidase (MAO), low plasma amine oxidase (AO), and high red cell catechol-O-methyl transferase (COMT) are related to adverse reactions to disulfiram. CSF DBH is inversely related to significant deviations in certain personality measures on the MMPI. Depressive ratings in alcoholics do not respond to disulfiram; depressive and aggressive affect are inversely related in alcoholics.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: (see Z01 MH 00092-10) Evidence obtained in recent years indicates that epinephrine (E), norepinephrine (NE), dopamine (DA), serotonin (5HT), acetylcholine (Ach), and gamma-amino-butyric acid (GABA), among others, act as neurotransmitters and/or neuromodulators of the central nervous system (CNS). Considerable indirect pharmacologic evidence has linked these amine systems with psychiatric illness (particularly affective illness and schizophrenia). Our work raises the possibility that searching for interrelationships between central biochemical functioning and repeated behavioral patterns may be more fruitful than searching for traditional diagnostic specificity of biochemical findings. In any case, further confirmation of relationships between central biochemistry and behavior could lead to more specific pharmacological treatments. Direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals. Only in recent years has data begun to accumulate on central neurochemical function in the various personality disorders. Some personality disorders, particularly those involving criminality, have patterns of a genetic component. Furthermore, certain patterns of behavior often seen within personality disorders; i.e., depression, alcoholism, and suicide, also appear to have genetic components. Data from animals suggests a relationship between aggressive behavior and neurotransmitters. Furthermore, this project is intended to contribute to a growing body of studies of human alcoholism. A purpose of this project is to extend the studies of central amine turnover into larger and more diverse populations of psychiatric patients and to assess behavioral-biochemical relationships and whether such findings are diagnostic specific. Dr. Frederick Goodwin continues to provide current scientific supervision on this multi-faceted project.

Methods Employed: The methodological details of two independent studies, both a separate effort of NIMH and a joint effort with the National Naval Medical Center, in Bethesda, MD and the Portsmouth Naval Medical Center in Portsmouth, VA, are provided in Z01 MH 00092-10. More recent analyses also include questionnaires of childhood behavior, school, and medical questionnaires completed by the subjects as adults. The use of standardized personality assessment instruments should facilitate attempts at further replication. All evaluative and behavioral data were collected, scored, and analyzed independently of the biochemical data.

Cerebrospinal fluid (CSF) was obtained from study group subjects following the procedures developed and revised at NIH and elsewhere. Assay details are described in the published studies. Other studies in conjunction with pharmacological interventions have further provided knowledge of functional brain chemistry in relationship to behavior, diagnosis, and personality.

Currently, clinical studies are planned to assess aggressive and suicidal behavior in subjects with histories of aggressive dyscontrol, alcoholics, affective disorders, schizophrenics, and normals. Of particular clinical interest is the interrelationship between aggression and suicide. These subjects will have their indoleamine metabolism assessed directly and indirectly in several ways; i.e., LP's, response to glucose tolerance testing (GTT) in alcoholics, hypothalamic serotonergic challenge (in normals), and a cross-over trial of carbamazepine and placebo in individuals with dyscontrol syndromes being followed by other physicians.

Among those individuals incarcerated for murder, responses to GTT and similar artificial sweetening will be assessed by the Thematic Apperception Test (TAT) along with baseline LP's in collaboration with Dr. Linnoila and colleagues.

A further study involves an assessment of serotonin and its metabolite from autopsy material in those individuals with a history of suicide and/or violence (in collaboration with Dr. Wyatt's group).

Major Past Findings: Initial results from personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment indicated that aggressive behavior is inversely correlated with 5-hydroxyindoleacetic acid (5HIAA) and positively correlated with 3-methoxy- 4-hydroxyphenylglycol (MHPG). Personality disorders have shown no significant difference in CSF cyclic 3',5-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from depressive, manic, and schizophrenic patients. Aggressive behavior was positively correlated with c-AMP and c-GMP in one group but not in a second. Those who were administratively discharged from the Service and those with history of suicidal attempts had lower CSF 5HIAA and higher MHPG, c-AMP, and c-GMP. Borderline personalities (DSM-III) showed an inverse relationship between CSF 5HIAA and the Pd scale, as well as a history of aggressive behavior; neither the MHPG relationships nor the cyclic nucleotide relationships were replicated. This study of c-AMP and c-GMP in borderlines has not yet been published. The trends are the same as those seen in the first study. Some of the differences between studies that may account for the non-replication of the MHPG and cyclic nucleotide findings are differences in diagnoses and homogeneity of intra-group behavioral patterns, smaller numbers of patients in the second study, and later refinements in assay methods. A trivariant relationship among a history of aggression, history of suicidal behavior, and lower CSF 5HIAA is readily apparent.

Of further interest is the initiation of new protocols (above) and the assessment of those accused of murder and who have a history of impulsive behavior. As our experience accumulates, the aggressive variable that appears to be most likely associated with lower CSF 5HIAA is that characterized by liability of affect, history of repeated impulsivity, and explosiveness. Similarly, our experience and that of others appears to indicate that suicidality associated with aggressivity is most likely to be associated with reduced levels of CSF 5HIAA. We intend to study individuals with histories of repeated suicidal behavior in themselves and their families.

A military male found guilty of violent murder, with a past history of several suicidal attempts, was found to have the lowest level of CSF 5HIAA yet measured by our group; he also had a hypoglycemic response to a GTT. In that aggressive behavior has been shown in animals to be associated with lower GABA, new studies of CSF GABA, both free and bound, have been analyzed in the borderline group of patients; though CSF GABA is lower in the more aggressive patients and in those with histories of suicidal behavior, neither difference reaches the $<.05$ level of significance. More patients would be needed to assess this preliminary finding.

Alcoholics do not differ from personality disorders in CSF HVA. However, mean CSF 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in abstinence to reach a mean level not differing from that of personality disorders. Although CSF HVA levels do not change post-intoxication-withdrawal, these levels

are depressed by disulfiram (Antabuse), a dopamine-beta-hydroxylase (DBH) inhibitor. Disulfiram use also correlates with an increase in serum NE. Mean serum DBH in alcoholics versus normal controls was lower, blood pressure was higher, and serum NE was not different. Disulfiram is also associated with an increase in cholesterol in alcoholics. Lower CSF DBH is correlated with increasing psychopathology, as measured by the MMPI, and lower CSF DBH is associated with disulfiram-induced psychoses. Furthermore, low platelet monoamine oxidase (MAO), low amine oxidase (AO), and elevated erythrocyte catechol-O-methyltransferase (COMT) are associated with disulfiram-induced psychoses. New studies show that neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism; nor can improvement in depression or anxiety ratings of hospitalized alcoholics be attributed to disulfiram.

The above represents studies that have been earlier published, those in press, and those in preparation. Continued collaboration with Dr. Linnoila of NIAAA involves the new protocols on aggression and suicidality, as well as collaboration on alcoholics to be admitted to NIAAA. New collaborations involve those with Dr. Wyatt's group at Saint Elizabeth's Hospital.

New Findings: Further analyses of previous studies indicate that those individuals diagnosed as anti-social and explosive (DSM-III) have the lowest levels of CSF 5HIAA; furthermore the MMPI profile of 42, 28, and 49 with high F scale scores are most closely associated with low CSF 5HIAA. The only Buss-Durkee Inventory (BDI) category that has a significant inverse relationship with CSF 5HIAA is "irritability". While total BDI scores and PD T scores do correlate significantly with a life history of aggression, the DBI appears to measure a number of aspects of aggressive thoughts and attitudes as well as behavior, but this scale appears to be a less useful instrument to relate to CSF 5HIAA levels. Attempts are now being made to follow the later life course of the original patients. A new finding not yet presented is the negative correlation between CSF 5HIAA (in the later group of Navy men) and a childhood history of conduct and attentional deficit disorder kinds of behavior. Medical history includes headaches as a child.

Significance to Mental Health Research: CNS functioning appears to be understudied in some major groups of psychiatric patients; viz, personality disorders, alcoholics, and borderlines. Studies of animal models, as well as Gilles de la Tourette's syndrome, hyperactive children, and prisoners suggest a relationship between central neurotransmitter systems and aggressive behavior. Human suicidal behavior has an enormous public health and social significance and, previously, had largely been studied from a psychological and sociological point of view. Both suicidal and aggressive problems are increasing. These studies lead to the possibility of identifying those at risk for anti-social and suicidal behaviors and possibly altering those behaviors through neuropharmacological adjuncts to management of the psychiatric and/or behavioral problems. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance in this prevalent problem.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The first lumbar puncture was performed in September 1974. This collaboration, however, continues to be of mutual benefit to NIMH and NNMC. Some neurochemical, behavioral, and psychological data are yet to be analyzed and reported from the patients who have participated in these studies, as well as the first attempts of a follow-up. New proto-

cols of a similar nature are being prepared, as described above, to continue this work within the BPB. Additionally, collaboration has begun on autopsy studies within the IRP at St. Elizabeth's Hospital.

Publications:

Brown, G.L., Goodwin, F.K.: Human aggression and suicide. In Maris, R. (Ed.): Biological Aspects of Suicide. New York, Human Sciences Press, in press.

Brown, G.L.: Serotonin: a focus for behavioral-biochemical data assessment from animals and humans. The Behavioral and Brain Sciences, in press.

Brown, G.L.: Biochemical alterations associated with aggressive behavior. In Bloomingdale, L.M. (Ed.): Attention Deficit Disorder. New York, Spectrum Publications, in press.

Brown, G.L., Goodwin, F.K.: Diagnostic clinical and personality characteristics of aggressive men with low 5HIAA. Clin. Neuropharmacology 7: 756-757, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00100-10 BP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biobehavioral Aspects in Childhood and Adolescent Mental Illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gerald L. Brown, M.D., Medical Officer, BPB, NIMH

Dr. Michael H. Ebert, Chairman, Department of Psychiatry, Vanderbilt University;
Dr. Judith Rapoport, Chief, Child Psychiatry, NIMH;
Dr. Alan J. Zametkin, Child Psychiatry, NIMH;
Dr. Dennis L. Murphy, Chief, Laboratory of Clinical Science, NIMH;
Dr. Robert M. Post, Chief, Biological Psychiatry Branch, NIMH

COOPERATING UNITS *(if any)*

Child Psychiatry Branch, Laboratory of Clinical Science, NIMH; Vanderbilt University

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	1.0	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This inpatient program with selected overnight stays for childhood and adolescent neuropsychiatric disorders largely involved those with conduct disorders and attentional deficit disorders. Pharmacological compounds studied were methylphenidate, amphetamine, piribedil, L-DOPA, tryptophan, Mianserin, clorgyline, and desipramine. Piribedil is safe but clinically ineffective in hyperactive children while L-DOPA is minimally clinically effective. Tryptophan is effective on attention measures. Pharmacokinetic studies with clinical responses are included. Amphetamine half-life in children is about one-third that of adults. Behavior and motor activity responses to d-amphetamine occur during the absorption phase as determined by serial plasma amphetamine following a single dose. Central neurotransmitters and their metabolites are being studied in plasma and urine. Urinary 3-methoxy-4-hydroxyphenylglycol (MHPG) shows a time-related decrease during treatment with d-amphetamine; dopamine metabolites are unchanged. Tyramine and its metabolites are also decreased following d-amphetamine, whereas phenylethylamine is greatly increased following d-amphetamine.

Project Description:

Objective: The purposes of this program are broad. An objective is to gain new knowledge of the central nervous system (CNS) of children and adolescents with special reference to maturational changes and neuropsychiatric disorders. Compared to the neurobiology known in adult neuropsychiatry, less is known regarding the neuropsychiatric disorders of children. A particular focus of these studies has been the relationship between neurotransmitter change in hyperactive children (HAC) following compounds that have major actions on central neurotransmitter metabolism. The study of d-amphetamine (d-AMPH), a compound with clear and reliable effects in HAC, has been of particular interest, its pharmacokinetics, its effects on catecholamine and indoleamine metabolism and on behavior, and the interrelationships of these effects.

There have been a number of hypotheses relating catecholamine metabolism and hyperactivity in children. The possibility of an overly active catecholaminergic system was first advanced. Later, a functional deficiency in catecholamines in HAC was proposed with the greater focus on the possibility of a functional dopamine (DA) rather than norepinephrine (NE) deficiency. Other biochemical alterations, particularly involving serotonin (5-HT) have also been proposed. More recently, alterations in phenylethylamine (PEA) have also been proposed. No single neurotransmitter system to date can be shown to have an etiological role. Dr. Michael H. Ebert and Dr. Judith L. Rapoport have provided overall collaboration and support for this multi-faceted project.

Methods Employed: An inpatient and day patient program for children and adolescents, involving selected overnight stays, was ongoing on an inpatient nursing unit. Children who were hyperactive, aggressive and impulsive, and who had learning difficulties, were admitted in order to study a carefully defined population of HAC. Children and adolescents with other conditions were also studied. Specific exclusion and inclusion criteria were employed.

All children were thoroughly evaluated by medical, psychiatric, and psychometric examinations with all routine and other indicated procedures and clinical laboratory studies. Children also received a psycholinguistic examination in collaboration with the National Institute of Neurological and Communicative Disorders and Stroke. Neurological examinations were scored carefully according to a rating scale (PANESS). Several clinical and behavioral rating instruments were utilized.

Pharmacological compounds, both standard and those previously unused in children, were studied. Serial plasma pharmacokinetic data was generated for d-AMPH. These data were studied in conjunction with motor activity, behavior cognition, speech, temperature, and cardiovascular response. Piribedil, a specific DA agonist, and L-DOPA were given to HAC. Mianserin, a NE agonist; tryptophan (TP), a precursor of 5HT; clorgyline, a monoamine oxidase inhibitor (MAOI); and desipramine, a tricyclic antidepressant; were administered to HAC in clinical trials.

Motor activity was measured by an ambulatory activity monitor with solid state memory which measured individual motor movements via pendulum acceleration system per unit of time and set at a desired sensitivity for the particular study. At any time the instrument could be read into a computer for a print-out. Behavioral changes in HAC were measured via Conners' Teachers' Rating Scale

(CTRS). Cognition was measured by a continuous performance task (CPT) in which errors of omission and commission could be scored in terms of differing time intervals. Time intervals could also be increased or decreased in relationship to accuracy of response.

d-AMPH was measured by radioimmunoassay (RIA) and gas chromatograph mass spectrometry (GC-MS). Biochemical studies include 24-hour urine collection to study NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), vanillymandelic acid (VMA), and noremetanephrine (NMN); DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxytryramine (3-MT); tyramine (TRM) and parahydroxyphenylacetic acid (PHPA); and phenylethylamine (PEA) and phenylacetic acid (PAA), in collaboration with Dr. Alan J. Zametkin. Plasma pharmacokinetics of pharmacological compounds were ascertained. Plasma NE, MHPG, NMN, and VMA changes as they relate to plasma d-AMPH levels have also been studied. Neurophysiological studies included routine and sleep EEG's and EMI scans when indicated. Averaged evoked response (AER) studies were conducted as they related to HAC in drug-free and treated conditions. Psycholinguistic changes were also studied in relation to d-AMPH, piribedil, and TP effects. Paired associate learning was also assessed in different drug conditions. Chronic effects of d-AMPH (2 weeks) were studied with regard to pharmacokinetics and clinical response, particularly in terms of evidence for tolerance or supersensitivity and effect on neurotransmitter metabolism as manifested by changes in plasma NE, MHPG, HVA, and dopamine-beta-hydroxylase (DBH) and platelet 5HT and MAO. The effects of TP and valine and d-AMPH and placebo were measured with regard to behavior, attention, rectal temperature, motor activity, and plasma amino acids and indoleamines.

Major Past Findings: Serial plasma pharmacokinetic data indicate that d-AMPH reaches a peak level in children within 3-4 hours of an initial dose; however, as much as 70-80% will remain in the serum at 5-6 h when behavioral effects have largely dissipated. Mean apparent elimination half-life is 6.8 ± 0.5 h. Test-retest studies of individuals indicate that both pharmacokinetic data and clinical response data are highly replicable. Sustained release capsules produce a slower rate of absorption and a more plateau-like, longer lasting peak level, but do not give a prolonged clinical response. Socially appropriate behavioral change and motor activity decrease is maximal at 103 h after administration of a single dose (0.5 mg/kg) of d-AMPH. Clinical changes may be related to a release of catecholamines and the subsequent depletion of their stores, replacement by "false" neurotransmitter metabolite of AMPH, or to alteration in receptor sensitivity. Higher single doses (1.0 mg/kg) effect earlier similar clinical response, but of less magnitude. Piribedil is safe but clinically ineffective in HAC. In one study, d-AMPH has also been shown to have an anti-aggressive effect in those HAC with a considerable degree of conduct disorder. In another recent study, whose report is still in preparation, preliminary results indicate that neither TP nor valine (a neutral amino acid which competes with TP and inhibits its crossing the blood-brain barrier) results in behavioral response or basal temperature change after a single dose, but attention span increase is similar to that observed following d-AMPH, while there are clear effects on plasma amino acids in the expected directions. This study was also designed such that the effects of the procedure itself could be accounted for. On the other hand, d-AMPH after a single dose appears to have no effect on serial plasma amino acids, 5HT, or 5-hydroxyindoleacetic acid (5HIAA) over a 6 h period. This preliminary finding could be quite important in that d-AMPH has been shown to

have clear effects on central 5HT in animals. Another recent study, also in preparation, indicates that both plasma MHPG and HVA are affected acutely by single-dose d-AMPH in a non-pretreated child, but this biochemical response may not be the same following two weeks of d-AMPH. Further analysis of this study may have implications for receptor change.

Urine studies indicate that day and night excretion of MHPG and HVA are not different; however, d-AMPH after 8 and 14 days is associated with lower MHPG levels. Behavioral response may be associated with the decrement in MHPG. Urinary HVA is unchanged. These biochemical and behavioral findings have been replicated in a subsequent HAC group, not yet published, as well as extended to other metabolites of both NE and DA. TRM and PHPA excretion are also decreased and PEA excretion is markedly elevated following two weeks of d-AMPH. PEA excretion is lower in HAC versus controls; its significance depends on whether it is expressed in terms of creatinine excretion. The TRM change may be associated with cardiovascular response and partially indicative of the change in PEA metabolism. More recent preliminary studies indicate a different pattern of metabolite response to methylphenidate (MP), a drug which produces a behavioral effect similar to d-AMPH. Though the effects are essentially opposite following MP with regard to NE and its metabolites, both d-AMPH and MP effect no change in DA or its metabolites.

HAC are not different from normals with regard to plasma NE and DBH but do have significantly more neurological soft signs by PANESS examination. New item analysis data indicates the prevalence of varied soft signs and their rater reliability. Plasma NE correlates with anxiety ratings and changes both with regard to dose of d-AMPH and time following dose, with higher doses of d-AMPH (1.0 mg/kg) giving strongest response at 1 hour and lower doses (0.5 mg/kg) giving strongest response at 3 hours. Elevated plasma NE is also associated with increases in blood pressure and pulse, and is dose-related. In a more recent study, baseline plasma NE, measured prior to an early a.m. dose of d-AMPH, does not change after two weeks of d-AMPH versus two weeks of placebo.

With regard to pharmacological response, d-AMPH is effective and piribedil and L-DOPA are minimally so; TP produces responses similar to d-AMPH. HAC with higher levels of soft signs have more abnormal EEG's, more minor physical anomalies, lower full-scale I.Q.'s (WISC-R), and a greater number of errors on the Bender. Data from psycholinguistic evaluations indicates that HAC have impairments in certain auditory processing and language skills; furthermore, d-AMPH does not evoke pronounced effects with regard to language performance in HAC vs. normals; older and less hyperactive subjects showed the most improvement. Improvement in cognitive parameters was shown only in normals.

New Findings: Platelet MAO is not significantly different in medication-free HAC vs. normals; AO is significantly lower in HAC vs. normals. MAO was not correlated with age in normal children (groups not different with age as a covariate); AO was not correlated with age in either group. MAO and AO levels were not related to a low monoamine diet platelet number, Hgb and Hct did not differ in the groups, nor was MAO or AO correlated with either. MAO and AO were determined two times in 20 hyperactives; the percent coefficients of variation (CV) were 18.6 ± 9.4 and 12.0 ± 9.2 respectively. Finally, neither MAO or AO responds significantly to d-AMPH.

Platelet 5HT, though not different in HAC vs. normals is negatively correlated with both attentional and conduct factors on the Connors Teachers-Rating Scale (CTRS), more strongly with conduct. These findings may explain the discrepant reports of 5HT in HAC when group data is compared to normals.

In view of the principal investigators interest in aggression, it is intriguing to report a case in which a very low level of CSF 5HIAA was found in a conduct-disordered adolescent, whose stealing involved a craving for sugar (glucose intake increases brain levels of 5HT). This individual also had an MMPI profile similar to that reported by Brown and colleagues for aggressive male adolescents with low levels of CSF 5HIAA.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly HAC, have been considerably studied in the last few years, there are many diagnostic, psychopharmacological and psychobiological questions yet to be answered. Many studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards HAC, obsessive-compulsive children, enuretics, Gilles de la Tourette's syndrome, anorexia nervosa, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and AMPH give positive responses in 80% of well diagnosed HAC, the pharmacokinetics and metabolism of these drugs have been studied only relatively recently. One avenue to ascertaining possible neuropathology in these conditions is to understand more clearly the mechanisms of action of these pharmacological compounds which effectively alter the clinical conditions under study. The relationship between such basic pharmacological knowledge and clinical effects has been under-studied in children in general, though the work of this group has greatly enhanced the knowledge of neuropharmacology and neurotransmitters in childhood mental illness. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in our understanding of childhood neuropsychiatry.

Proposed Course of Project: The principal investigator, Dr. Brown, remains in the Office of the Chief, BPB, and is no longer administratively a part of the Child Psychiatry Branch, Testing of the last active subjects from the general inpatient project was completed during the spring of 1983, though collaboration continued in some instances. There is a body of data yet to be analyzed but some of this is in preparation and in press and will be reported in the future. Dr. Robert M. Post, Chief, BPB, provides collaboration and support.

Publications:

Zametkin, A.J., Brown, G.L., Karoum, F., Rapoport, J.L., Chuang, L.W., Langer, D.H., Wyatt, R.J.: Urinary phenylethylamine response to d-amphetamine in boys with attention deficit disorder. Am J. Psychiatry, 141:1055-1058, 1984.

Brown, G.L., and Ebert, M.H.: Catecholamine metabolism and hyperactive children. In Lake, C.R. and Ziegler, M.G. (Eds.): The Catecholamines in Psychiatric and Neurological Disorders. Boston, London, Buttersworth Publishers, 1985, pp. 93-130.

Brown, G.L., Ebert, M.H., Minichiello, M.D.: Biochemical and pharmacological aspects of attention deficit disorder. In Bloomingdale, L.M. (Ed.): Attention Deficit Disorder. New York, Spectrum Publishers, 1985, pp. 93-130.

Zametkin, A.J., Karoum, F., Linnoila, M., Rapoport, J.L., Brown, G.L., Chuang, L.W., Wyatt, R.J.: Stimulant, urinary catecholamines and indoleamines in hyperactivity: a comparison of methylphenidate and dextrosamphetamine. Arch. Gen. Psychiatry, 42:251-255, 1985.

Brown, G.L.: Attention deficit disorder. In Kagan, B.M. and Gellis, S.S. (Eds.) Current Pediatric Therapy, W.B. Saunders, Phiadelphia, PA, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00070-12 BP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychological and Biological Interactions in the Mood and Anxiety Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert M. Post, M.D.	Chief	BP NIMH
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Dr. W. Mendelsohn	Clinical Psychobiology Branch, NIMH	
W. Duncan	Clinical Psychobiology Branch, NIMH	
Dr. H. Holcomb	Lab. of Psychology & Psychopathology, NIMH	
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Dr. L. DeLisi	Clinical Neurogenetics Branch, NIMH	

COOPERATING UNITS *(if any)*

CNG,NSB,NPB,CPB,LCM,LCS,LPP,RSB,IRP, NIMH; DEB, NICHD; DPCBR, NIAAA; PDS, NIH; USUHS, Dept of Def.; U. of CA; VA Med. Center, Bronx; Tufts U.; Thos. Jefferson Univ., Walter Reed Med. Cntr; USC Med. Sch.; U. So. Carolina Med. Sch.; INSERM

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12.0	6.0	6.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical evaluation, research, and treatment of patients with manic-depressive illness, schizoaffective disorders, and anxiety disorders are the primary goals of the Section. Double-blind, placebo-controlled clinical trials are employed to evaluate routinely used and novel agents for the treatment of these disorders. A number of anticonvulsants are being employed, one of which - carbamazepine - has been demonstrated by our group to be clinically effective in the acute and prophylactic treatment of manic-depressive illness. We are seeking to identify clinical and biochemical markers of response to carbamazepine and other agents, and to elucidate the mechanisms of action of these drugs. Recent data suggest that noradrenergic and "peripheral-type" benzodiazepine mechanisms may be important to the anticonvulsant effects of carbamazepine; they may also be important to its psychotropic properties. However, like lithium carbonate, carbamazepine has a multitude of effects on a variety of neurotransmitter, modulator, and peptide substances, many of which may account for positive effects on mood and behavior. The Section also seeks to identify its regional alterations in brain electrophysiological and metabolic activity and relate these changes to behavioral and cognitive changes in affective illness. A clinical probe of limbic system excitability utilizing a novel provocative agent, procaine, is also being employed. Preliminary data suggest that this drug selectively increases fast activity over the temporal lobe in association with a variety of behavioral and cognitive alterations and secretion of cortisol, ACTH, and prolactin.

Animal models of electrophysiological kindling, stimulant-induced behavioral sensitization, and unavoidable stress or "learned helplessness" are studied by the Section. The behavioral relevance of these models to clinical disorders is also explored, as well as possible basic neurophysiological and biochemical mechanisms which might underlie these long-term changes in behavior.

COLLABORATORS:

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I. Project Description

A. Objectives

This project is engaged in the multidisciplinary longitudinal study and treatment of patients with a spectrum of acute and chronic psychoses, particularly involving mood and anxiety disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their interaction.

B. Methods Employed

1. Subjects who meet Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness or the more recent DSM III criteria for a spectrum of mood disorders are admitted to the 3-West Clinical Research Unit, Section on Psychobiology of the Biological Psychiatry Branch. Patients with anxiety and panic-anxiety are also admitted to the unit under other protocols (see Project Z01 MH 00071-05 BP). Normal volunteers are admitted to the unit to provide control data for specific studies in patients and to assess clinical and biological interrelationships in normal as well as patient populations.

2. Psychological and Biological Evaluation

a. Behavior and Cognition: During an initial drug-free interval patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation, including EEG-monitored sleep, averaged evoked potentials, and a variety of cognitive tests. These include the Halstead Category Test, a psychosensory questionnaire, a neuropsychological profile using the Luria Test, and an extensive battery of tests developed in collaboration with P. Brouwers and A.F. Mirsky of the Laboratory of Psychology and Psychopathology. Longitudinal behavioral data are collected in a double-blind fashion utilizing twice-daily global ratings by nurses trained as observers. Patients also complete twice-daily ratings of mood and side effects in order to assess subjective effects and examine diurnal variation. Using the same double-blind methodology, nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) three times weekly.

b. Life Chart Methodology: A life chart technique has been developed to plot the number and severity of affective episodes and the interval between episodes so that the longitudinal development, recurrence, and progression of the illness can be accurately quantified and illustrated. Patients are followed up, outside of the hospital, at regular intervals to assess the efficacy of treatment interventions.

c. Physiology: Motor activity is measured continuously at 15-minute intervals with a miniaturized activity monitor developed by Dr. T. Colburn. EEG-monitored sleep is studied in collaboration with Dr. W. Mendelson and W. Duncan. In collaboration with Drs. R. Coppola, H. Holcomb, and M.S. Buchsbaum, 16-channel EEG's averaged evoked responses are conducted.

d. Functional Anatomy: In addition to computerized axial tomography (CAT-scan) evaluation of our patients for possible cerebral pathology, studies have been initiated in collaboration with Drs. R. Cohen, L. DeLisi and M.S. Buchsbaum and associates to study regional functional activity of the brain using (18F) flurodeoxyglucose.

e. Urinary Free and Plasma Cortisol, Dexamethasone Suppression and CRF Stimulation: Basal 24-hour urinary free cortisol is measured during depressed and manic states in medication-free conditions and during treatment. A detailed evaluation of the pituitary-adrenal axis is conducted by Drs. D.R. Rubinow and P.W. Gold in patients with affective illness and anxiety disorders. Plasma cortisol is measured under basal conditions and following the dexamethasone suppression test. Hormonal response to CRF before and during treatment with carbamazepine is studied with Dr. P.W. Gold.

f. Cerebrospinal Fluid (CSF) and Plasma Studies: Plasma and CSF studies comprise an important area of biological evaluation of classical neurotransmitters and their amines, as well as the newly discovered peptide substances, in normal volunteers and in patients during ill and well intervals. These studies are conducted in collaboration with Drs. D.R. Rubinow, P.W. Gold, D.C. Jimerson, and M. Linnoila, as well as many investigators within and outside of NIMH, with specialized techniques for measurement of specific peptide hormones. GABA levels in CSF of affectively ill patients and controls are measured in collaboration with Dr. T. Hare.

g. Oxytocin and Vasopressin: In collaboration with Drs. H. Weingartner, P.W. Gold, and D.R. Rubinow, infusions of these peptides are utilized to assess effects on memory, mood, and endocrine function in affectively ill patients and normal volunteers.

h. Procaine Activation: Procaine, an agent which activates limbic system structures with some selectivity, is administered intravenously in graded doses to affectively ill, borderline, and normal subjects to assess possible altered behavioral, electrophysiological, or biochemical responsivity in this system. Collaborators include Drs. C. Kellner, M. Kling, F. Putnam, J. Chassey, R. Coppola, D. Gardner, and R. Cowdry.

3. Treatment

Treatment and evaluation are conducted in individual and group therapy, and ongoing clinical case conferences are utilized. Both routine and experimental compounds are evaluated during double-blind clinical trials to study clinical efficacy and mechanisms of action. The routinely used drugs include tricyclic antidepressants, lithium carbonate, monoamine oxidase inhibitors, and neuroleptics.

New treatments include the anticonvulsant carbamazepine which is evaluated for its acute and prophylactic efficacy, clinical and biological predictors of response, and mechanisms of action. Diphenylhydantoin and valproic acid are two other anticonvulsant agents also being studied in selected patients. Clonidine, an alpha-2 agonist, is administered during clinical trials to assess the clinical efficacy of alterations in adrenergic functioning in anxiety and affective illness.

The paradoxical antidepressant effects of one night's sleep deprivation in depressed patients are explored both to develop a model for further understanding the rapid onset and offset of a non-pharmacologically-induced mood improvement and to assess its therapeutic potential.

4. Animal Models.

A rodent behavioral pharmacology laboratory is maintained in collaboration with Drs. S. Weiss and A. Pert to develop new research techniques in several areas. The longitudinal evolution of behavioral pathology is assessed using a number of paradigms including: 1) electrophysiological kindling; 2) pharmacological kindling; 3) behavioral sensitization to psychomotor stimulants and related dopaminergic agonist compounds; and 4) the evaluation of stress sensitization and learned helplessness and their possible underlying neural substrates. Physiological and biochemical changes, particularly alterations in receptor binding, are studied in collaboration with Drs. A. Pert, P. Marangos, J. Patel, and D. Jacobowitz. The role of seizures in the development of behavioral pathology is studied utilizing kindling and CRF. The anticonvulsant mechanism of action of carbamazepine is also studied in the kindling paradigm.

C. Major Findings

1. Carbamazepine: A New Treatment for Manic-Depressive Illness

This year, Dr. Post received the Gold Medal Award of the Society of Biological Psychiatry for his work on carbamazepine and its theoretical underpinnings.

a. Acute Antimanic Efficacy: In collaboration with Drs. T. Uhde, R. Joffe and other physicians in the Branch, we have documented unequivocal evidence of the efficacy of carbamazepine in the acute treatment of manic episodes, including many patients who were previously nonresponsive to lithium carbonate. Twelve of 19 acutely manic patients have shown good responses: those who were the most manic at the onset responded best. Rapid cyclers (who are often non-responders to lithium) responded well; good responders averaged seven episodes of illness in the year prior to admission, while poor responders to carbamazepine had three. In an "off-on-off-on" design where carbamazepine and placebo are administered in an alternate fashion, and with nurses "blind" to medication status, we have noted repeated clinical improvement during carbamazepine treatment and exacerbation during placebo substitution. The time-course of improvement on carbamazepine parallels that of neuroleptics.

b. Acute Antidepressant Efficacy: Twenty-two of the first 37 patients have shown evidence of clinical response to carbamazepine. In 12 patients, marked clinical improvement was observed. Patients with initially more severe depression responded better to carbamazepine than those with less severe ratings of depression. Family history of affective illness did not predict response, but acute antidepressant response to sleep deprivation did predict subsequent response to carbamazepine. Those with more rapid cycling (episodes/years ill) and hospitalizations for mania, but fewer total weeks depressed (i.e., less chronic depression), also responded better. Baseline motor activity profiles, studied by Dr. R. Joffe, did not predict carbamazepine response, although motor activity increased in the evening hours in responders.

c. Prophylactic Efficacy of Carbamazepine: Nine patients have been followed for a mean of 2.7 years on carbamazepine in either a double-blind or an open fashion with carbamazepine as a single treatment or as an adjunct. In these lithium-nonresponsive, rapidly cycling manic-depressive patients, carbamazepine decreased the mean number of affective episodes per year from 17.0 in the years prior to carbamazepine treatment to 6.5 episodes/year on the drug. The severity

and duration of episodes when they did occur were also reduced on carbamazepine. Discontinuation of the drug resulted in relapses in five of six patients, further indicating that improvement was related to carbamazepine and not to spontaneous improvement in the course of illness.

d. Side Effects: The drug is well tolerated in the majority of patients, with mild and clinically insignificant decreases in white count observed in the majority of patients. No patient had to be dropped from the trial because of a low white count. Rashes were observed in 10-15% of patients, requiring drug discontinuation. Mild decreases in serum sodium and calcium are also observed, as documented by Drs. R. Joffe and T. Uhde. Sedation and dizziness are dose-related and tend not to occur with slow increases in dose. The effects of carbamazepine on thyroid function elucidated by Drs. P. Roy-Byrne and R. Joffe have led to a major reconsideration of the role of thyroid hormones in affective illness (see section h,2 below). While daytime sedation is not a problem, substantial improvement in sleep has been noted in half-hour sleep checks by nurses blind to active carbamazepine administration. In the first 27 depressed patients, sleep significantly increased ($p < .001$) during the first week of carbamazepine. This often preceded clinical improvement in depressed mood and was maintained during the clinical trial. Similarly, in the first 19 manic patients studied, sleep almost doubled in the first week of carbamazepine administration ($p < .01$).

e. Plasma and CSF Levels of Carbamazepine and its -10,11-Epoxide Metabolite: Spinal fluid levels of carbamazepine and its 10,11-epoxide metabolite are measured in collaboration with Drs. T.W. Uhde and P.K. Narang. Carbamazepine levels in plasma or in CSF (a measure of free carbamazepine) were not significantly related to degree of clinical antidepressant or antimanic response and preliminary evidence of a significant relationship to levels of carbamazepine-10,11-epoxide is being studied in a larger series of patients. However, our data suggest the possibility that the 10,11-epoxide metabolite, which we and others have noted to have anticonvulsant effects in animals, may also possess active psychotropic properties in man.

f. Comparison with Other Anticonvulsants: Clinical trials have been initiated to examine the relative efficacy of carbamazepine in comparison to other anticonvulsants such as phenytoin and valproic acid. In the first patient to complete a double-blind crossover design, no evidence of clinical improvement was observed with phenytoin or valproic acid, while the patient was an unequivocal carbamazepine responder. These data suggest the possibility that biochemical or physiological properties peculiar to carbamazepine may, at least in this patient, be important to its psychotropic properties rather than relating to generalized anticonvulsant effects. Emrich and associates in Europe, and investigators in five other countries, have, however, reported the successful use of valproic acid in a small number of lithium-resistant manic-depressive patients.

Although carbamazepine is a highly effective anticonvulsant, it is also useful in the treatment of a variety of paroxysmal pain syndromes which clearly do not involve an ictal process. Thus, the efficacy of carbamazepine does not imply that subclinical seizures are the underlying pathophysiological mechanism in patients with affective illness. However, the properties mediating carbamazepine's anticonvulsant effects may nonetheless be related to its psychotropic properties. The clinical utility of the anticonvulsant carbamazepine raises the paradox of

why the major motor seizures of electroconvulsive therapy are among the most effective treatments for acute manic and depressive illness. As detailed below, we have observed that electroconvulsive seizures in the rat are paradoxically anticonvulsant to amygdala-kindled seizures. These data raise the possibility that common biochemical and physiological mechanisms of electroconvulsive therapy and the anticonvulsant carbamazepine could be related to their profile of therapeutic efficacy in both phases of affective illness.

g. Studies of Carbamazepine's Mechanism of Action:

1) Effects on Classical Neurotransmitters and Modulators: Evidence in laboratory animals (Purdy et al.) suggests that carbamazepine blocks the reuptake of norepinephrine (NE) but also inhibits stimulated-induced release. We have observed, in collaboration with Dr. D.C. Jimerson, that carbamazepine treatment significantly reduces the NE metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), in CSF of patients with affective illness. CSF NE itself, measured in collaboration with Drs. C.R. Lake and M. Linnoila, is not significantly altered in the depressed patients; however, the elevated levels of CSF NE in mania are decreased by carbamazepine. Noradrenergic effects of carbamazepine have been linked to its anticonvulsant properties. We have observed that the alpha-2 antagonist yohimbine reverses the anticonvulsant effects of carbamazepine on amygdala-kindled seizures.

There is substantial evidence that carbamazepine does not act as a classical neuroleptic and does not block dopamine receptors. It does not block cocaine- or amphetamine-induced hyperactivity or stereotypy and does not raise HVA levels in rat brain or in the spinal fluid of our patients with affective illness, as do the classical neuroleptic treatments. Moreover, it has not been associated with the development of parkinsonian side effects or with the syndrome of tardive dyskinesia as have the neuroleptic drugs. Carbamazepine produces only slight increases in serum prolactin. These data suggest that carbamazepine acts by mechanisms other than blockade of dopamine receptors.

Alterations in GABA have been postulated in affective illness (see below) as well as in the seizure disorders. Carbamazepine has been reported to decrease the turnover of GABA in animal studies (Bernasconi, 1984), although brain levels are not altered by the drug. This is consistent with our data indicating that CSF GABA levels are not significantly decreased during treatment with carbamazepine compared to baseline levels. d-Baclofen does not reverse the anticonvulsant effects of carbamazepine, as it does in models of trigeminal neuralgia, suggesting that $GABA_B$ mechanisms are not critical to carbamazepine's anticonvulsant actions.

Effects of carbamazepine on central and "peripheral-type" benzodiazepine receptors have been studied with biochemical techniques (Marangos et al.), and electrophysiologically in the amygdala kindling model. Carbamazepine binds poorly to the central site (^{3}H-diazepam or ^{3}H-BCCE), but more potently at the Ro5-4864 (peripheral) site. Dr. S.R.B. Weiss has found that Ro-15-1788 and BCCM block the anticonvulsant actions of diazepam, but are ineffective in reversing the anticonvulsant effects of carbamazepine on amygdala kindling. Conversely, Ro5-4864 does reverse the anticonvulsant effects of carbamazepine and its 10,11-epoxide, but not those of diazepam. PK11195, an antagonist at the peripheral site, blocks the effects of Ro5-4864 against carbamazepine, further supporting an effect through this mechanism. Taken together, these biochemical and electrophysiological data sug-

gest the possibility that carbamazepine may have physiologically relevant effects on the Ro5-4864 site in brain.

Carbamazepine is potent in displacing binding of several adenosine receptor ligands (Marangos et al.). Contrary to predictions, chronic treatment with carbamazepine (similar to that with caffeine) increased the number of adenosine receptors, suggesting that carbamazepine may possess some adenosine antagonist-like properties.

2) Carbamazepine's Effects on Endocrine and Peptide Systems: Carbamazepine significantly decreased somatostatin measured in CSF of affectively ill patients in collaboration with Drs. D.R. Rubinow, P.W. Gold, and S. Reichlin. These findings are of interest in relationship to the report of long-lasting increases in brain somatostatin following amygdala kindling seizures and the findings of low CSF somatostatin in depressed patients. These findings could relate to the anticonvulsant properties of carbamazepine as others have recently demonstrated that blocking somatostatin function inhibits seizures.

Carbamazepine's effects on vasopressin are noteworthy from both a clinical and theoretical perspective. In contrast to lithium carbonate, which produces the diabetes insipidus syndrome, carbamazepine has been used to treat diabetes insipidus. It has antidiuretic properties which are manifest by its effects in producing mild hyponatremia (Drs. R. Joffe and T.W. Uhde). During carbamazepine treatment, decreased endogenous vasopressin is secreted in response to a hypertonic saline load, also consistent with an agonist role in this system (Drs. P.W. Gold and J.C. Ballenger). These findings are opposite those observed during lithium carbonate treatment. Dr. W.H. Berrettini has documented that carbamazepine is the one psychotropic drug tested to date that displaces ^{125}I-arginine vasopressin binding from platelets, further suggesting that carbamazepine may have direct effects at the vasopressin receptor.

The effects of carbamazepine on cortisol are noteworthy from several perspectives. Rubinow and associates have found that carbamazepine induces escape from dexamethasone suppression, even in depressed patients who are showing clinical improvement. Urinary free cortisol excretion is also increased by carbamazepine. Carbamazepine may thus be affecting regulation of the pituitary-adrenal axis directly, or through its effects on higher neural substrates in the limbic system or elsewhere.

The possible effects of carbamazepine on endogenous opiate systems are of interest in relation to the efficacy of carbamazepine in pain syndromes and the fact that it potentiates opiate-induced running activity in mice. Carbamazepine did not alter CSF opiate binding activity in 17 affectively ill patients, or affect morphine's antinociceptive effects on tail flick latencies in the rat.

In contrast to lithium, carbamazepine inhibits rather than potentiates the TSH response to TRH (Drs. R. Joffe and P.W. Gold). Like lithium, carbamazepine decreases plasma levels of T_3, T_4, and free T_4 in a highly significant fashion, but it does not result in a marked increase in basal TSH or in clinical hypothyroids, as may occur with lithium. Drs. P. Roy-Byrne and R. Joffe have found, paradoxically, that those patients with the greatest decrease in T_4 and free T_4

were the best responders to carbamazepine. This has led Dr. Joffe to a new conceptualization of the role of thyroid hormones in affective illness (see below).

Continued study of carbamazepine's biochemical effects, either alone or in comparison with lithium carbonate, may ultimately prove useful not only in further understanding its mechanism of action in affective illness, but also in helping to understand substrates underlying the affective disorders.

2. Approaches to Neurotransmitter-Receptor Dysfunction in Affective Illness

a. Norepinephrine: Consistent with its effects on decreasing firing of the noradrenergic locus coeruleus in animals, clonidine acutely decreased plasma NE and MHPG, measured in collaboration with Drs. T.W. Uhde, C.R. Lake, and D.C. Jimerson. Dr. Uhde found that clonidine was associated with anti-anxiety effects measured on the Spielberger Rating Scale in depressed and anxious patients but not in normal volunteers. Clonidine's effects are consistent with the observations that CSF NE (Dr. R. Lake) and CSF MHPG (Dr. D.C. Jimerson) may be slightly elevated in some depressed patients (possibly those with greater anxiety) compared to normal volunteers. However, CSF NE is markedly increased in manic patients compared to either of the other patient or control populations. In addition to the state-related alterations in noradrenergic function, we have been interested in assessing the relationship of this system to the longitudinal course of affective illness, as assessed by life chart methodology. Several findings suggest that increases in noradrenergic function measured during an acute episode of depression may be positively related to greater frequency of cycling as well as poorer prognosis variables.

b. GABA: Indirect pharmacological data support a possible role of GABA in affective illness. Moreover, direct measurements of GABA in plasma and CSF provide some evidence of disturbed GABA function (Drs. Berrettini, Joffe, and Hare). These studies, suggesting possible GABA alterations in affective illness, are of interest in relation to recent reports that GABA agonists may have antidepressant effects, and that several agents reported to be effective in the treatment of recurrent affective illness (electroconvulsive therapy, lithium, carbamazepine, and valproic acid) all decrease GABA turnover.

c. Dopamine: Indirect biochemical, pharmacological, and endocrine data continue to suggest a role for dopamine in some aspects of affective illness. Dopamine and its metabolite HVA and DOPAC are studied in collaboration with Drs. D. Rubinow, M. Linnoila, and R. Joffe in depressed, manic, and euthymic patients and controls. Preliminary studies of the relationship of plasma HVA to the longitudinal course of affective illness do not suggest as close a relationship, to mood as to the level of psychosis.

3. Thyroid Function in Affective illness

Based on the findings that responders to carbamazepine showed greater decreases in T_4 and free T_4 than non-responders, R. Joffe examined other pharmacological data suggesting similar relationships. He found that three different treatments effective in mania and depression (carbamazepine, lithium, and ECT) all paradoxically appeared to alter peripheral indices of thyroid function in the direction of relative hypofunction. How might this be compatible with consistent and growing evidence that T_3 potentiates that antidepressant

response to tricyclics? Dr. Joffe noted that T_3 treatment, by decreasing circulating T_4, may also be inducing relative CNS thyroid hypofunction. In contrast to the periphery which is able to directly use T_3, 80% of the brain's T_3 is derived from T_4. Thus, T_3 potentiation may be a misnomer and may be acting by decreasing rather than increasing thyroid indices (especially T_4).

4. Peptides in CSF: Interrelationships with Neurotransmitter and Behavioral Alterations

Most neuropeptide substances that have been suggested as putative CNS neurotransmitters or modulators are measurable in CSF. In many instances there is evidence that CSF levels are regulated independently of those in the periphery. The CSF thus provides one strategy for attempting to identify peptidergic alterations in neuropsychiatric disorders and to examine their postulated relationship to alterations in behavior, cognition, and affect. Neuropeptides have recently been reported to co-exist in the same neurons with classical neurotransmitter substances. Again, the CSF provides an opportunity for studying the potential interaction between both classical neurotransmitters and the recently discovered neuropeptides.

CSF somatostatin has been measured in the CSF of affectively ill patients and normal volunteers by sensitive radioimmunoassay in collaboration with Drs. D.R. Rubinow, S. Reichlin, and P.W. Gold. Dr. Rubinow found that CSF somatostatin was significantly decreased in depressed patients compared to those re-studied in the euthymic state or compared to normal volunteer controls. There have now been five replications of the finding of low somatostatin in depressives compared to normals or other psychiatric control groups. These findings are of interest in relationship to the reports of decreased somatostatin in brain and CSF of patients with Alzheimer's disease and several other neuropsychiatric syndromes that can present with cognitive defects. As noted above, carbamazepine significantly decreased CSF somatostatin, while lithium and other psychotropic drugs produced no significant alterations and zimelidine significantly increased CSF somatostatin. These findings thus open new areas for exploration of the possible role of somatostatin decreases in depression and in the possible mechanism of action of carbamazepine. Dr. Rubinow, in collaboration with Drs. Doran, D. Pickar, A. Roy and S. Paul has also found that depressed and schizophrenic patients who were cortisol hypersecretors, as indicated by escape from dexamethasone suppression, had significantly lower CSF somatostatin.

Dr. Rubinow received the A.E. Bennett Award from the Society of Biological Psychiatry for his work on somatostatin.

Drs. P.W. Gold and D.R. Rubinow have further studied pituitary-adrenal dysregulation in affective illness. They have observed significantly higher excretion of urinary free cortisol in unipolar and bipolar depressives compared to normal volunteers, with significantly lower levels in manic patients. These findings are paralleled by a large literature of well-documented and replicated studies indicating that approximately 50% of depressed patients show evidence of cortisol hypersecretion measured either by escape from dexamethasone suppression, increased urinary free cortisol, or altered diurnal variation of cortisol secretion. In addition, Dr. Rubinow has documented marked state-related alterations in urinary free cortisol secretion and highly significant correlations in 8 AM plasma cortisols with severity of depression in cycling manic-depressive patients studied longitudinally. Higher levels of urinary free cortisol correlated with

greater cognitive impairment on the Halstead Categories test of abstracting ability. These data are of some theoretical relevance, as well as of possible clinical significance, since depressed patients often have marked complaints of subjective decreases in cognitive and memory capacity.

Dr. Gold has completed a series of studies of CRH infusions in affectively ill patients and controls (as described in Project # Z01 MH 00452-10 BP) and found evidence for blunted ACTH response in depressive but not manic or improved states. In contrast to depressed patients, hypercortisolemic patients with Cushing's disease show ACTH hypersecretion to CRF, providing a possible differential diagnostic test. Last year, Dr. Gold, in collaboration with Dr. Chrousos, was recognized for this pioneering work with the receipt of the Curt Richter Prize in Psychoneuroendocrinology.

Drs. Rubinow, Gold, and Weingartner are studying the effects of infused oxytocin and vasopressin on mood and cognitive capacities of affectively ill patients and normal volunteers. Initial data suggest that vasopressin enhances, while oxytocin impairs, certain aspects of cognition and that vasopressin increases cortisol secretion.

Drs. Uhde and Bierer have found that caffeine significantly increases cortisol levels following dexamethasone, suggesting an important potential dietary artifact in the widely used dexamethasone suppression test.

5. Life Charting the Course of Affective Illness

In collaboration with Dr. P. Roy-Byrne, Dr. T.W. Uhde, A. Rosoff and D. Davis, we have recently completed the first phase of analysis of the life course of illness in 95 unipolar and bipolar patients. The recurrent nature of affective illness in our patient population is again emphasized by the finding that 80% of the patients relapsed within five years of their first episode. Fifty-five percent relapsed within the first year of that episode. The hazards of making a diagnosis of unipolar depression are also reemphasized in our sample, as we have found that eight of 23 bipolars (34%) had not yet had their first manic episode after three or more depressive occurrences. However, 21 of 23 depressed patients (92%) had converted to bipolarity after six depressive episodes. We also found that a shorter well interval between the first and second episode predicted an increased number of total weeks ill subsequently. Several mood phases or switches within the first episode, as opposed to an isolated mania or depression, also predicted increased total number of weeks ill. Bipolar patients who presented with a first depression showed a relatively greater proportion of depressions to manias subsequently, tended to have a greater proportion of depressions preceding mania, and were more likely to become rapid cyclers. Twenty-four of 46 (56%) bipolar patients showed a pattern of sensitization characterized by an increasing rapidity of cycling and decreased well interval between successive episodes of affective illness; these patients had more episodes in the year prior to NIMH admission, a higher percentage of depressive compared to manic episodes, and an increased frequency of hospitalizations for depressions.

It was particularly noteworthy that, compared to unipolars, bipolar patients had increased numbers of total and depressive episodes, increased episodes per years ill, and increased episodes in the year prior to NIMH admission, yet they showed an equal amount of total time ill. This emphasizes the finding that

bipolar patients tend to have more episodic and cyclic courses to their illness. Interesting findings did emerge comparing bipolar female and male patients. Females showed an increased number of hospitalizations for depression. Moreover, the 34 bipolar females showed a pattern of more rapid cycling (5.8 ± 1.1 episodes in the year prior to NIMH admission) when compared to the 23 male bipolars (2.7 ± 0.5 episodes prior to NIMH admission; $p < .04$). These data are consistent with other studies in the literature suggesting that female patients are more prone to rapid cycling illness and are of considerable interest in relation to theories of underlying neurotransmitter and hormonal alterations in females compared to males. Rapid compared to less rapid cycling bipolar patients showed an increased number of weeks ill in the year prior to NIMH hospitalization as well as increased number of hospitalizations for depression. The occurrence of rapid cycling also predicted an increased number of weeks hospitalized at NIMH (43 wks compared to 27 wks for non-rapid cyclers) as well as more manias and total episodes of affective illness observed at NIMH. Thus, rapid cycling prior to NIMH admission correlated with a pattern of continued rapid cycling during NIMH admission.

The study of the longitudinal course of affective illness provides a template not only for assessing the phenomenology of the illness and its response to treatment interventions with agents such as lithium and carbamazepine, but also refocuses on possible biological mechanisms underlying the recurrent and, at times, progressive aspects of affective illness. For example, we have found increased CSF norepinephrine and GABA levels in depression are associated with greater rapidity of cycling.

We suggest that the life charting process is a useful clinical as well as research tool and may help to focus on possible environmental precipitants and dynamically significant events and stresses that may be temporally related to affective episodes. It also allows precise characterization of the degree of longitudinal response to newly available pharmacological agents. Recent data of Wehr and Goodwin have emphasized that some pharmacological interventions, such as the tricyclic antidepressants, may actually result in increased rapidity of cycling. The life chart methodology provides a useful instrument for documenting and intervening in this problematic side effect.

6. Menstrually-Related Mood Dysfunction (Dr. D.R. Rubinow)

A relationship between mood and behavior and menstrual function has been described with respect to a number of disorders including premenstrual tension, post-partum depression, epilepsy (so-called catamenial epilepsy), and menopausal dysphoria. Dr. D.R. Rubinow has initiated a series of studies to investigate the relationship between mood disorders and the menstrual cycle (see project #Z01 MH 00180-03 BP). These studies include: development of a questionnaire which is being employed to help determine the incidence and nature of affective symptoms in relation to the menstrual cycle; assessment of the precision of the relationship between mood changes and the menstrual cycle utilizing daily self-ratings and daily temperature recordings; investigation of hormonal activity employing periodic blood samples and neuroendocrine tests; and assessment of the efficacy of progesterone, a synthetic progestin, and carbamazepine in the treatment of established menstrually-related mood syndromes. The results of such a study may: 1) determine whether a specific association between depressive symptoms and menstrually-related phenomena (menstruation, post-partum depression, menopause, hormone-induced behavioral change) can be established; 2) reveal the incidence of the entrainment of depres-

sive symptoms to the menstrual cycle; 3) help elucidate the nature of the "switch" mechanism in affective disorders and periodic psychosis; and 4) determine the efficacy of pharmacologic agents believed useful in the treatment of menstrually-related mood disorders. For example, Dr. Rubinow has found that only 43% of self-referred patients have prospectively documented menstrually-related mood disorder; none of the first ten patients studied showed a positive response to progestational agents.

7. Depressive Subtypes and Symptoms in Relation to Regional Localization of Function

a. Psychosensory Phenomena: In collaboration with Drs. E.K. Silberman, J-P. Boulenger, L. Bierer and T.W. Uhde, we have developed an interview rating scale designed to measure signs and symptoms that are usually associated with psychomotor epilepsy (complex partial seizures). We have studied these phenomena in patients with primary affective illness and panic-anxiety illness, in patients with documented evidence of temporal lobe epilepsy, and in a medical control group of hypertensive patients. Compared to the control group, patients with affective illness, panic-anxiety disorders, and with epilepsy showed a highly significant increased incidence in the number of these signs and symptoms. The qualitative symptom profiles differ slightly among the affective, anxious, and epileptic patients. Depressed patients with a history of panic attacks have more symptoms than depressed or anxious patients without panic attacks and show a profile highly characteristic of panic patients. To the extent that psychosensory distortions and related symptoms usually associated with temporal lobe epilepsy are occurring with a high incidence in patients with primary affective illness, these data might suggest that some of the neural substrates involved in complex partial seizures overlap with affective illness. Affective patients with greater numbers of psychosensory symptoms responded better to lithium carbonate, and preliminary data suggest that this is not the case for carbamazepine, as we would have predicted.

b. Psychological, Structural, Metabolic, and Electrophysiological Approaches to Regional Brain Function in Affective Illness: A variety of psychological test batteries are employed to assess possible alterations in regional brain function in patients with affective illness, including the Luria Battery, the Halstead Categories Test, and other cognitive tests studied in collaboration with the Laboratory of Psychology and Psychopathology. Consistent with patients' subjective sense of cognitive impairment during depression, marked impairment in cognitive function has been documented on the Halstead Categories Test. Degree of cognitive dysfunction correlated with increases in urinary free cortisol secretion, suggesting that cortisol hypersecretion may be primarily or secondarily related to this important subjective and objective deficit in depressed patients.

Computerized axial tomography (CAT) scans have been performed on our patients with affective illness and reveal a similar range of increased ventricular brain ratios (VBRs) comparable to those observed in schizophrenic patients. We are currently assessing the clinical and biological concomitants of this evidence of altered brain structure in a subgroup of affectively ill patients with Dr. C.H. Kellner. Dr. Kellner observed that patients with the greatest urinary free cortisol excretion had the largest VBRs. These data are consistent with those in the literature indicating that treatment with ACTH or exogenous glucocorticoids such as dexamethasone is associated with reversible atrophy and enlarged ventricles on

CAT scan. This literature and our findings suggest that "structural" alterations in brain on the CAT scan may not be as irreversible as previously thought and that exogenous and perhaps endogenous biochemical changes may be important mediators of this brain measure which is receiving increasing attention as a possible concomitant of some patients with schizophrenic illness.

Twenty-one medication-free affectively ill patients also showed a significant relationship between VBR and degree of cognitive impairment measured on the Halstead Categories Test (with Drs. Kellner and Rubinow). As described in detail elsewhere, topographic mapping of EEG frequencies and averaged evoked response is being conducted in collaboration with Drs. R. Cohen, L. DeLisi, H. Holcomb and M.S. Buchsbaum. These studies, in conjunction with positron emission tomography (PET) scan studies, may provide important evidence of electrophysiological and/or metabolic regional dysfunction in affective illness. Initial studies indicate that acutely ill and improved affective disorder patients show a nonspecific pattern of hypofrontality similar to that observed in schizophrenia and other patient populations. These and other data indicate that relative hypofrontality in glucose utilization is not specific to the psychopathology of schizophrenia. Other alterations more intimately related to affective symptomatology remain to be elucidated. Glucose utilization in temporal cortex relative to other areas in the same brain slide was lower in depressed patients compared to controls. These data provide evidence that depressed patients differ from patients with active complex partial seizures who show areas of increased glucose utilization in the temporal lobe (Engel et al., 1982) and further suggest that the anticonvulsant carbamazepine (see above) is not acting by dampening covert seizure activity in our patients with affective illness.

d. Procaine Infusions as a Probe of Limbic System Responsivity: Graded doses of the local anaesthetic procaine were administered to affectively ill patients (in collaboration with Drs. C. Kellner, F. Putnam and M. Kling), borderline personality disorders (in collaboration with R. Cowdry and D. Gardner), and normal volunteers in an attempt to probe limbic system responsivity. Analysis of the first 21 subjects by Dr. R. Coppola reveals increases in fast EEG activity, especially 26 to 45 Hz, selectively over the temporal cortex, confirming in man the suggestions from animal studies that local anaesthetics activate temporal lobe and limbic structures. Dose-related alterations in subjective sensory and cognitive functions were reported as well as a variety of affective responses ranging from mood elevation to dysphoria. Vivid recall of experientially immediate memories, as well as hallucinatory-like phenomena, occurred less often. In patients with borderline personality disorder, degree of fast activation of the temporal cortex was not positively correlated with response to carbamazepine. In collaboration with Drs. P. Gold, C. Kellner, and M. Kling, procaine-induced release of ACTH, cortisol, and prolactin, but not growth hormone, has also been documented. These data suggest the utility of procaine as a potential pharmacological probe of the limbic system and temporal lobe.

8. Laboratory Studies of Behavioral Sensitization and Electrophysiological Kindling (in collaboration with Dr. S.R.B. Weiss)

a. Stimulant-induced Behavioral Sensitization: We have investigated the phenomenology and mechanisms underlying the increased behavioral responsivity to the same dose of the psychomotor stimulant cocaine. Animals adminis-

tered cocaine (10 mg/kg i.p.) once-daily show increasing amounts of locomotor hyperactivity and stereotypy to the same dose over time. Brattleboro homozygote rats lacking vasopressin showed deficient onset, maintenance, and persistence of cocaine-induced behavioral sensitization compared to their heterozygote littermate controls. We have replicated the original findings and further show that vasopressin replacement will reverse the deficit in cocaine-induced behavioral sensitization. Female rats, compared to male rats, are more responsive to the same dose of cocaine. They demonstrate similar behavioral sensitization to repeated injections of cocaine at approximately half the dose (5 mg/kg) used in males (10 mg/kg, i.p.). An environmental context and conditioning component has been demonstrated. For example, animals repeatedly treated with cocaine in the context of the test cage showed greater degrees of hyperactivity and stereotypy than animals receiving identical doses in a different environment. These findings have been replicated using drug or saline injections into the nucleus accumbens; cocaine pretreated animals showed an increased response to intracerebral saline or amphetamine only when treated in the same environment.

b. Electrophysiological and Chemical Kindling: Repeated, intermittent electrical stimulation of the brain results in increasing duration, spread, and complexity of electrical after-discharges culminating in the appearance of major motor seizures to a previously subthreshold stimulation. We have employed this procedure in order to study long-lasting changes in neural and behavioral excitability that accompany this process. Following electrical kindling of the amygdala, rats showed decreased spontaneous and cocaine-induced exploratory activity, while they showed increased convulsive susceptibility to a local anaesthetic, lidocaine. Repeated injections of the same dose of lidocaine (65 mg/kg, i.p.) also lead to an increasing incidence, severity, and duration of seizures to the same dose over time. Repeated lidocaine-induced seizures sensitize to electrophysiological kindling of the amygdala. These data suggest cross-sensitization between electrical and lidocaine-induced pharmacological kindling.

Behavioral alterations (irritability and resistance to capture) persist in the interictal period following lidocaine-induced seizures. In collaboration with Drs. L. Sokoloff, C. Kennedy and their associates in the Laboratory of Cerebral Metabolism, it has been demonstrated that lidocaine-induced seizures relatively selectively increase metabolic activity in limbic system structures, particularly amygdala, hippocampus, perirhinal, and cingulate cortical areas. This paradigm would therefore appear to be a useful one in exploring the relationship of seizures with some specificity for limbic structures to alterations in irritable and aggressive behavior.

Studies with Dr. S.R.B. Weiss have shown that carbamazepine (15 mg/kg, i.p.) is a potent inhibitor of completed amygdala-kindled seizures, but at this dose is not effective in suppressing the development of kindling in the rat. We have demonstrated that the metabolite carbamazepine-10,11-epoxide is also effective in inhibiting amygdala-kindled seizures, although it is slightly less potent than carbamazepine itself.

Possible mechanisms underlying the kindling process itself are being studied in collaboration with Drs. Weiss, Patel and Marangos. Preliminary evidence has been obtained indicating that 24 hours following kindling there is selective phosphorylation of a 45K protein in the amygdala bilaterally; it is not observed

following repeated ECT seizures. Dr. Patel has extended these findings with the observation of regional changes in phosphorylation. He found changes in a calcium-calmodulin sensitive 35K protein bilaterally in the amygdala of kindled rats. Additional proteins, including an 87K protein (SMP) and an 80K (synapsin) also appeared to be increased by kindling. These changes were not observed with lidocaine-induced seizures, suggesting some specificity to the process. A series of studies are being conducted to replicate and extend these findings.

c. Electroconvulsive Shock Inhibits Amygdala Kindling: It appears paradoxical that carbamazepine (an anticonvulsant) and electroconvulsive therapy (i.e., major motor seizures) are both effective in the treatment of manic-depressive illness. We have found that the major motor seizures of electroconvulsive shock (ECS) are themselves anticonvulsant to amygdala-kindled seizures. In fact, ECS is more potent than carbamazepine in inhibiting the development of kindling. These data raise the possibility that the efficacy of electroconvulsive therapy in patients with affective illness could be related to effects mediating its anticonvulsant actions. As reviewed above, we have used the kindling model to study the anticonvulsant mechanisms of carbamazepine and evidence suggests that an alpha-2 and a "peripheral" benzodiazepine effect are of importance.

d. CRF Seizures and Behavior: Interaction with Amygdala Kindling: Dr. S.R.B. Weiss, in collaboration with Drs. A. Pert and P. Gold, has conducted a series of studies on the behavioral and convulsive effects of corticotropin releasing hormone (CRF) administered intracerebroventricularly. CRF induces the late onset (i.e., following a lag 4-6 hours post injection) of seizures that behaviorally and electrophysiologically resemble those produced from electrical stimulation of the amygdala. Following repeated once-daily administration, tolerance develops to the effects of CRF on seizures. Despite this, CRF seizures enhanced the development of amygdala-kindled seizures such that animals pretreated with CRF develop electrically kindled seizures twice as fast as vehicle-injected controls. CRF-treated animals also show increases in aggressive behavior toward other rats.

The convulsive response to CRF was not reliably reproduced by local intracerebral injection into amygdala, hippocampus, septum, hypothalamus, or PAG. However, the aggressive behavior could be elicited by CRF injections into PAG. Moreover, small lesions of the amygdala decreased the CRF-induced aggression following i.c.v. administration, but did not affect the development of seizures. Lesions of the hippocampus and olfactory tubercle similarly did not block the development of seizures produced by i.c.v. CRF. These data suggest that CRF is inducing seizures highly similar to those produced by electrical stimulation of the amygdala, but that they are not dependent on an amygdala substrate for their occurrence. Further, these data suggest the possibility that an endogenously produced, stress-related peptide such as CRF may, under pathological conditions, be associated with alterations in convulsive and aggressive responsivity.

D. Proposed Course of Project

We have helped to introduce and document carbamazepine as an effective treatment modality for manic-depressive and schizoaffective illness; we propose to further delineate clinical and biological markers of carbamazepine response. Preliminary evidence suggests that many patients who clearly do not respond to

lithium carbonate will respond to carbamazepine. It will be increasingly important to establish whether response to carbamazepine, compared to lithium carbonate, delineates separate subgroups of patients with affective illness, such as those with rapid-cycling illness. The degree of generalization of carbamazepine response to other anticonvulsant agents such as phenytoin or valproic acid will be another area of both clinical and theoretical import. This is also particularly the case in light of our recent findings that electroconvulsive shock exerts potent anticonvulsant effects on limbic system seizures. Are anticonvulsant effects of a variety of treatment modalities linked to therapeutic response in affective illness? Carbamazepine is clearly useful in pain syndromes that do not involve a convulsive process, and effectiveness of anticonvulsant agents in a subgroup of patients with affective illness does not imply an underlying ictal process. The possible mechanisms of action of carbamazepine in our patients, as well as in behavioral pharmacological models, will also be pursued. We will investigate whether carbamazepine's anticonvulsant metabolite, carbamazepine-10,11-epoxide, also has important psychotropic properties in manic-depressive patients.

The interrelationship of classical neurotransmitter substances with the putative CNS neurotransmitter peptides will be explored in both patients with affective illness and anxiety disorders, in collaboration with Dr. T.W. Uhde. A variety of techniques are in place for measurement of neurotransmitter and receptor function in both classical neurotransmitter systems and in the peptide systems in man. These will be correlated with behavioral alterations and changes in mood and cognitive functioning in patients with mood and anxiety disorders. Particular focus will be given to studies of CRF to elucidate its utility in the differential diagnosis of cortisol hypersecretion of Cushing's disease and depression (Gold and associates). Alterations in somatostatin as they relate to affective and seizure mechanisms will also be systematically explored, especially in light of growing evidence of alterations in somatostatin in depression and in a variety of neuropsychiatric disorders.

As described in detail in Project # Z01 MH 00071-05 BP, Dr. T.W. Uhde will continue to explore the similarities and differences in patients with panic anxiety syndromes and those with affective illness in terms of acute symptomatology, longitudinal course of illness, and response to pharmacological agents. Catecholamines appear to be altered in both the mood disorders and in panic anxiety disorders. Response to treatments which act on catecholamine systems such as clonidine will be compared and contrasted in both patient populations. The clinical utility of carbamazepine will also be explored in panic anxiety syndromes. Since caffeine has been shown to induce escape from dexamethasone suppression, the clinical, mechanistic, and theoretical implications of this important observation will be systematically followed up by Dr. Uhde and his associates.

Dr. D.R. Rubinow is continuing to study and treat patients with menstrually-related exacerbation of mood and behavior disorders. He will be examining this problem from a clinical and endocrinological point of view, and as a model for studying the acute onset and offset of affective dysfunction.

Work in animal models will continue to focus on possible mechanisms underlying behavioral sensitization and electrophysiological kindling. In collaboration with Drs. S.R.B. Weiss, P. Marangos, and J. Patel, neurotransmitter receptors, protein phosphorylation, and ion channels will be examined as possible mediators

or modulators of the electrophysiological kindling paradigm. The mechanisms of anticonvulsant action of carbamazepine on amygdala-kindled seizures will also be further studied. Studies of behavioral and biochemical response to repeated stress (avoidable and unavoidable) will be performed in collaboration with Drs. S.R.B. Weiss and A. Pert. The role of environmental context and conditioning will also be examined in these paradigms.

E. Significance to Biomedical Research and the Program of the Institute

Based in part on work in this Branch, carbamazepine has emerged as a new treatment for manic-depressive illness. Carbamazepine's importance is further highlighted by the fact that it is effective in some patients who do not respond to lithium carbonate. Studies of the mechanism of action of carbamazepine may provide new leads to the understanding of mechanisms of action of other effective antimanic and antidepressant drugs as well as basic mechanisms underlying affective dysregulation. Study of endocrine and peptide substances in man and animals may also provide new conceptual and practical treatment approaches to the relationship between manic and depressive symptoms and biochemistry. Examination of the interaction between classical neurotransmitters and the peptides should prove fruitful in understanding normal and pathological functioning. The multi-disciplinary assessment of our patients' mood, behavior, cognition, physiology, and biochemistry should allow more precise characterization of important biobehavioral relationships and their underlying neural substrates. Elucidating the mechanisms underlying behavioral sensitization and kindling, which appear to involve processes akin to memory, may provide important information regarding the coding of behaviorally relevant long-term changes in the CNS. Thus, basic and clinical research have led to important findings in neurobiology and the development of a new treatment for affective illness with carbamazepine. This year, Drs. Rubinow and Post received the two research awards of the Society for Biological Psychiatry for their clinical research findings on somatostatin and carbamazepine, providing further evidence of the recognition of this work and its potential clinical import.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00072-05 BP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychophysiological Investigation of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Frank W. Putnam, M.D., Staff Psychiatrist, Adult Psychiatry Branch, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been incorporated into Project #Z01 MH 002270-02 NP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00071-05 BP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Psychobiological Correlates and Treatment of Panic and Related Mood Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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LAB/BRANCH
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INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with pathological degrees of anxiety who meet DSM III criteria for agoraphobia with panic attacks or panic or phobic disorders are evaluated using psychological, physiological, and biochemical methodologies. Patients with major affective illness, particularly those with a significant anxiety component, are also eligible for participation in the program. Particular attention is given to the role of the noradrenergic neurotransmitter system as assessed by: 1) measurement of the metabolite MHPG in urine and plasma; 2) adrenergic receptor number and function in platelets; and 3) neuroendocrine and behavioral response to the alpha-2 adrenergic agonist clonidine, and antagonist yohimbine. Research investigating the relationship of noradrenergic function to other neurotransmitter systems and the hypothalamic-pituitary-adrenal axis also has been initiated. Caffeine and nifedipine challenges are administered to assess their behavioral and biochemical effects. Other approaches to understanding the pathophysiology of anxiety and its potential treatment with alprazolam, carbamazepine, clonidine, imipramine and verapamil will be explored.

PHS 6040 (Rev. 1/84) GPO 904-917

COLLABORATORS:

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L. Bierer, M.D.	Medical Staff Fellow, BPB, NIMH
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E. Klein, M.D.	Visiting Associate, BPB, NIMH

I. Project Description

A. Objectives

This project employs a multidisciplinary team in the study and treatment of pathological anxiety, major affective and related mood disorders.

B. Methods Employed

1. Subjects

a. Patients who meet Research Diagnostic Criteria for panic, phobic, and generalized anxiety disorders, as well as patients who meet DSM III criteria for major affective illness, are candidates for participation in the project. Inpatients are studied and treated on the 3-West Clinical Research Unit and outpatients are followed through the Ambulatory Care Research Facility. A number of previously validated scales to measure state and trait anxiety are utilized and an analogue anxiety scale and panic anxiety scale have been developed to more adequately assess the relationship among state anxiety, phobic anxiety, avoidance behavior, and depressive symptomatology.

b. Normal volunteers are also accepted into the project to provide control data, as well as to assess the relationship between normal state anxiety and selected psychological and biological variables.

2. Psychological and Biological Evaluation

a. Baseline Evaluation. During an initial evaluative period patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. This initial evaluation is indicated due to the heterogeneous nature of the panic and phobic disorders. Anecdotal reports suggest that many medical illnesses may present as or exacerbate pre-existing conditions of pathological anxiety. However, no research has systematically studied a large number of panic and phobic patients to determine the incidence and prevalence of these associated disorders.

b. Life Chart Methodology. A life chart technique has been developed in collaboration with Dr. J.-P. Boulenger, Dr. P. Roy-Byrne, M. Geraci, and Dr. L. Bierer to plot the frequency, intensity, and interval between panic attacks. The character and the change in the quality of panic attacks is assess as a function of duration and longitudinal course of illness. This approach will allow us to document the development, recurrence, and progression of the panic and phobic disorders. Life charting is an important aspect of the overall project because few systematic studies have been conducted on the natural progression of these disorders.

c. Sleep and Sleep Deprivation. Electroencephalographic sleep recordings are obtained for three consecutive nights. Although many panic anxious patients, like endogenously depressed individuals, have improved sleep following treatment with tricyclic and monoamine oxidase inhibitors, little is known about the sleep architecture of panic and phobic anxious patients. The

effects of one night's sleep deprivation on mood and behavior are investigated in patients with panic disorder and major depressive disorder.

d. Insensitivity Index. Using threshold and signal detection methodology, an index of pain insensitivity is obtained in panic patients and normal volunteers. The effects of traditional and novel antianxiety agents will be studied with this paradigm.

e. Galvanic Skin Response. The effects of alprazolam and imipramine and selected anxiogenic agents (e.g., yohimbine) on physiological measures of galvanic skin response, reaction time to auditory tones, pulse, and respiratory rate are studied in panic and phobic anxious patients and age-matched normal volunteers. This investigation is performed in collaboration with Drs. T. Zahn and M. Albus.

f. Echocardiography. Echocardiography is obtained in patients and age-matched controls to assess the presence or absence of mitral valve prolapse. These data are obtained in collaboration with Drs. E. Tucker and S. Palmeri who are blind to the diagnosis of each patient or normal volunteer when echocardiography and auscultation are performed.

g. Computerized Axial Tomography. Cerebral CAT Scans are obtained, and, in collaboration with Dr. C. Kellner, cerebral ventricular size is determined in patients with panic disorder. Enlargement of the cerebral ventricles has been reported in schizophrenic and affectively ill patients.

h. Psychomotor Activity. Twenty-four hour motor activity is assessed with a miniaturized activity monitor worn on the wrist of patients with primary anxiety disorders and age- and sex-matched normal controls.

i. Caffeine. Caffeine is administered to panic patients and normal controls to assess behavioral and biochemical responses to this agent whose effects are thought to be mediated through the adenosine, GABA-benzodiazepine, and noradrenergic systems.

j. Clonidine -- An Alpha-Adrenergic Agonist. Clonidine is administered intravenously to anxious and affectively ill patients and normal volunteers to assess clinical, physiological, and neuroendocrine responses to this noradrenergic drug.

k. Yohimbine -- An Alpha-Adrenergic Antagonist. Yohimbine is administered in an oral challenge to panic anxious and affectively ill patients and normal controls to assess the clinical and biochemical effects of this noradrenergic antagonist which is known to potently increase noradrenergic function in the animal.

l. Nifedipine -- A Calcium Channel Blocker. Nifedipine is administered orally to agoraphobic patients exposed to phobic situations to determine the antianxiety effects, if any, of calcium channel blockers.

m. Urinary MHPG and Urinary Free Cortisol. Amine metabolites and urinary free cortisol are systematically evaluated using daily 24-hour urine collections across clinical state changes on and off medication.

n. Dexamethasone Suppression Test. Dexamethasone is administered to patients to evaluate the pituitary adrenal axis. Basal values are performed at baseline and at 8:00 am, 4:00 pm, and 11:00 pm following dexamethasone administration.

o. Urine and Plasma Studies. Amine metabolites, electrolytes, and peptides are also measured in the urine and blood.

p. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function as well as prostaglandin-stimulated increases in cyclic-AMP are assessed in patients and age-matched normal volunteers.

q. Glucose Tolerance Testing. In collaboration with Dr. B. Vittone, clinical and metabolic parameters are evaluated following the oral administration of glucose.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual and/or group supportive sessions. In addition, ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. Standard medications used for the treatment of pathological anxiety may be used and include tricyclic antidepressants, monoamine oxidase inhibitors, minor tranquilizers, and beta-blockers.

C. Major Findings

1. Medical Illnesses and Anxiety

Detailed physical, neuropsychiatric, and laboratory evaluations continue to be performed in patients admitted to our program. As reported last year (Z01 MH 00071-04 BP), 69% of our panic patients had previously undiagnosed medical illnesses. Although these illnesses appeared to be unrelated to the direct pathogenesis of the panic attacks themselves, these data extend previous research suggesting that psychological (major life events) and physiological (medical illnesses) stressors may trigger panic attacks in biologically vulnerable individuals.

In an attempt to further define the characteristics of brain structure in panic disorder, we continue to investigate, in collaboration with Dr. C. Kellner, the cerebral ventricular size (VBR) in agoraphobic patients with panic attacks. Although patients with panic disorder tend to have a normal mean VBR

($\bar{x}$ = 3.6 ± 2.5), our data continue to suggest a significant positive correlation between duration of benzodiazepine use and VBR.

2. Psychosensory Symptoms

In collaboration with Drs. L. Bierer, J.-P. Boulenger, and E. Silberman, we have investigated the occurrence of 52 psychosensory symptoms typically associated with partial complex seizures. In addition, we have investigated the differential distribution of these symptoms in affectively ill patients with and without a positive history of panic attacks. Results confirm and extend our earlier observations that patients with affective disorders experience increased psychosensory symptoms (derealization, depersonalization, perceptual changes, time distortions, etc.) in during episodes of illness. Increases in psychosensory phenomena were reported by patients with panic disorder (n = 64), affective illness (n = 81), and temporal lobe epilepsy (n = 37) when compared to normal (n = 90) or medical (n = 30) controls. Affective patients with panic attacks, however, experienced significantly greater numbers of symptoms than either the affectives without panic attacks or panic disorder patients. The profile of symptoms reported by each patient group differed, and affective patients with panic attacks demonstrated a symptom profile characteristic of affective illness combined with that of panic disorder. That several sensory and cognitive illusions were associated significantly more frequently with panic attacks than with psychomotor seizures is one provocative finding of this study.

3. Life Course of Illness

The life course of panic disorder continues to be assessed retrospectively in all patients with panic attacks. While the retrospective study of psychiatric illnesses has a number of methodological flaws, this study represents one of the first attempts to obtain an accurate understanding of the longitudinal course of panic disorder using systematic life chart methodology. As reviewed in greater detail in last year's report (Z01 MH 00071-04 BP), several preliminary conclusions can be made regarding the phenomenology and longitudinal course of panic disorder. First, the onset of panic attacks generally begins in adolescence or early adulthood and, if untreated, frequently leads to an impaired life style characterized by pathological degrees of anticipatory or free-floating anxiety and agoraphobia. Second, lifetime symptoms of major depression occur in approximately 50% of the patients, although only 24% of all panic disorder patients develop longstanding endogenomorphic symptoms of depression. Third, tricyclic antidepressants appear to have antipanic effects independent of the presence of concomitant depressive symptomatology.

4. Effects of Diazepam on Cognition and Psychophysical Pain

In collaboration with Drs. P. Roy-Byrne and H. Holcomb, the effects of a single 10 mg oral dose of diazepam versus placebo on cognition, psychophysical pain, and mood was assessed in ten normal males. Diazepam impaired attention and effort-demanding cognitive processing without affecting more superficial processing or subjects' awareness of the accuracy of learning

and recall. It produced significant increases in drowsiness, dreaminess, and decreases in the sense of not being able to think clearly. Finally, it reversed the usual improvement in sensory discrimination seen on successive signal detection trials. These effects on mood, cognition, and psychophysical pain were not correlated within individuals.

5. Sleep and Sleep Deprivation

Although insomnia is commonly believed to result from anxiety or other states of increased autonomic arousal, few studies have systematically investigated the sleep architecture of patients with panic disorder or agoraphobia with panic attacks. In last year's annual report (Z01 MH 00071-04 BP), we reported in detail the preliminary findings on the sleep EEG of panic disorder patients. Since a major focus of the Unit's ongoing research is the investigation of the relationship between panic and major depressive disorders, we were particularly interested in the nature of rapid-eye-movement (REM) parameters in patients with panic disorder compared to normal controls. Although the panic patients had a shorter mean REM latency, it was nearly twice as long as that typically reported in patients with endogenous depression. The number of REM periods and % of REM sleep were not different between the panic patients and normal controls. Moreover, the panic patients demonstrated a normal progression of increasing REM sleep during the course of the night, a pattern frequently opposite that found in melancholic depressed patients. When compared to normal controls, panic disorder patients would appear to be different from patients with major depressive disorder, melancholic subtype. Since REM deprivation has been associated with a transient antidepressant response, our preliminary sleep EEG findings demonstrating apparent differences in REM sleep between panic disorder and major depressive disorder led our Unit to study the behavioral effects of one night's total sleep deprivation (therefore, including REM deprivation) in panic disorder patients compared to depressed patients and normal controls. In collaboration with Drs. P. Roy-Byrne and R. Post, the effect of one night's sleep deprivation, a method which has been described in detail elsewhere (Arch. Gen. Psychiatry), on mood and behavior was evaluated in 12 patients with panic disorder, ten depressed patients, and ten controls.

In contrast to the improvement in symptoms of anxiety and depression shown by the majority of depressed patients, the majority of panic disorder patients showed noticeable worsening in their symptoms of anxiety, with 40% experiencing panic attacks on the day following sleep deprivation. EEG recordings with nasopharyngeal electrodes on the day following sleep deprivation were normal, suggesting that panic disorder patients do not have seizure activity characteristic of temporal lobe epilepsy. Thus, our preliminary data suggest that panic disorder patients do not have the same proclivity as depressed patients to respond positively to one night's sleep deprivation. These findings, submitted to the Arch. Gen. Psychiatry , together with our previous data demonstrating a different pattern of REM sleep published in Psychiatry Research, suggest important differences between panic disorder and depressed patients in sleep-related physiology and clinical responsivity.

6. Caffeine: Behavioral and Biochemical Effects

Several studies have been conducted by the Unit on Anxiety and Affective Disorders to investigate the behavioral and biochemical effects of caffeine in panic disorder patients and normal controls. The following section reflects the scientific rationale and chronological sequence of our research with caffeine.

a. Caffeine: Retrospective Survey. As previously reported (Z01 MH 00071-04 BP), a caffeine-consumption survey was designed in collaboration with Dr. J.-P. Boulenger and administered to patients with panic and major depressive disorders and compared to normal controls matched for age, sex, and socioeconomic status. Data from this survey, published in the Arch. Gen. Psychiatry, indicated an increased sensitivity to the psychostimulant and anxiogenic effects of caffeine in panic disorder patients compared to their normal controls. This relationship was not found in the patients with major affective disorders. The findings of this survey, suggesting an increased vulnerability to the anxiogenic effects of caffeine in patients with panic disorder, led us to directly test the single-dose behavioral and biochemical effects of caffeine in panic disorder patients and normal controls. To pursue this goal, our Unit first investigated the effects of three separate doses of caffeine in normal controls.

b. Caffeine: Dose-related Behavioral and Biochemical Effects in Normal Controls. Using double-blind, placebo-controlled conditions, three doses of oral caffeine (240, 480, and 720 mg) were administered to 12 normal controls. Caffeine produced dose-related increases in state anxiety (ANOVA, $p < .004$), mean arterial pressure (MAP) ($p < .007$), plasma lactate ($p < .002$), and plasma cortisol ($p < .00001$). Plasma NE and its principal metabolite, MHPG, failed to increase. Of interest, two of 12 normal controls developed unequivocal panic attacks following the 720 mg dose of caffeine. Moreover, these two panicking normal controls had a greater than five-fold increase in mean cortisol (peak concentration minus baseline value, $\bar{x} = 15.8$ μg/dl ± 1.9 SE) after caffeine 720 mg, compared with the increase in cortisol ($\bar{x} = 2.8 \pm 2.2$) ($p < .05$) in the nonpanicking normal controls. This research, conducted in collaboration with Drs. J.-P. Boulenger, D. Jimerson, W. Potter, M. Linnoila, and S. Sinclair and Ms. M. Geraci, demonstrated that caffeine in sufficient doses may induce anxiety, including panic attacks, in normal subjects. The lack of caffeine's effects on MHPG further suggested that noradrenergic systems might be responsible for the major psychostimulant effects of caffeine in euthymic humans.

c. Increased Sensitivity to Caffeine in Panic Disorder Patients. To directly test our hypothesis that panic patients have an increased vulnerability to the anxiogenic effects of caffeine, a caffeine dose (480 mg) which failed to elicit panic attacks or severe degrees of generalized anxiety in the normal controls, was administered under double-blind, placebo-controlled conditions, to 24 panic disorder patients and 14 normal controls. This study, conducted in collaboration with Drs. J.-P. Boulenger and L. Bierer, did support our hypothesis of increased sensitivity to caffeine in panic patients, as indicated by a significantly greater increase in measures of anxiety on the Zung

Anxiety Scale in the patients compared to normal controls ($p < .01$). Moreover, nine of 24 panic patients, but none of the 14 normal controls, experienced panic attacks by DSM III criteria. Compared to normal controls, the panic patients also had significantly higher levels of cortisol ($p < .02$), lactate ($p < .02$), and glucose ($p < .01$) following caffeine, although only increased levels of lactate distinguished between panicking and nonpanicking patients. It should be underscored that the normal controls did have significant increases in both measures of anxiety ($p < .01$) and plasma cortisol ($p < .001$), compared to their placebo control condition. Thus, while panic patients appear more sensitive to the anxiogenic effects of caffeine, normal subjects are not insentient to the psychostimulant properties of caffeine.

d. Alprazolam Blocks Anxiogenic Effects of Caffeine. In collaboration with Dr. L. Bierer, we have conducted preliminary studies investigating the effects of alprazolam, a triazolabenzodiazepine with antipanic properties in humans, on caffeine-induced anxiety. Blinded caffeine 480 mg was administered to patients participating in a double-blind, alprazolam-placebo crossover study. While six of 16 (37.5%) on the placebo phase of the study had panic attacks following single dose caffeine (480 mg), none of 11 (0%) of the alprazolam-treated patients had panic attacks following this same acute oral dose of caffeine ($p = .027$, Fisher's exact test). Of interest, alprazolam blocked the usual caffeine-induced increment in lactate but had no effect on plasma cortisol levels. These behavioral and biochemical effects suggest that the benzodiazepine receptor system may play an important role in blocking some of caffeine's psychostimulant and biochemical effects. The role of the GABA-benzodiazepine receptor system in mediating caffeine's principal panicogenic effects remains to be elucidated.

e. Caffeine-induced Escape from Dexamethasone Suppression. The dexamethasone suppression test (DST) has been suggested as a sensitive and specific tool for the diagnosis of major depressive disorder, melancholic subtype. Because psychiatric patients have been reported to consume excessive amounts of caffeine, because caffeine produces dose-related increases in plasma cortisol (refer to 6b, 6c of this report), and because the effects of caffeine (probably the most widely consumed psychotropic agent the world) on the DST had not been previously reported in the literature, the single-dose effects of caffeine 480 mg on the standard dexamethasone suppression test was investigated in 22 normal volunteers and six depressed patients. Using a single-blind design, an oral dose of caffeine 480 mg or placebo was administered randomly on two separate days at 2:00-2:30 pm the day following the 11:00 pm administration of dexamethasone 1 mg. Test days were separated by at least 48 hours. Blood samples were obtained at 4:00 pm.

Caffeine significantly increased the post-dexamethasone cortisol values. Whereas the 4:00 pm cortisol values after placebo averaged 2.3 ± 2.3 (mean ± SD), the comparable mean value after caffeine was 5.3 ± 5.8 (paired $t = 3.7$, $p < .001$). A plasma cortisol level of > 5 μg/dl has been used most commonly to signify nonsuppression. Of the 28 subjects, four (14%) were found to be nonsuppressors on placebo and ten (36%) were nonsuppressors on caffeine. Caffeine-induced nonsuppression was observed in both depressed patients and normal volunteers. This study, conducted in collaboration with Drs. L. Bierer

and R. Post, is the first investigation to our knowledge demonstrating that escape from dexamethasone suppression can be induced by caffeine. Of interest, the 480 mg single dose of caffeine given to subjects in this study is roughly comparable to four to five cups of coffee and within the range typically consumed on a daily basis by 20%-40% of the population. Since several lines of evidence suggest that psychiatric patients, particularly depressed and schizophrenic patients, may consume excessive amounts of caffeine, our findings may explain in part the wide variability and discrepant findings in the literature on the DST in psychiatric patients. This major new finding, published in the Arch. Gen. Psychiatry, will require the scientific community to re-evaluate the degree to which caffeine and other dietary factors influence the DST and other neuroendocrine tests. At the very least, caffeine should now be added to the list of substances and conditions that can induce "false positives" on the DST.

7. Glucose Tolerance Testing

In collaboration with Dr. B. Vittone, we continue to investigate glucose metabolism in patients with panic disorder and normal controls. We previously reported (Z01 MH 00071-04 BP) that panic patients given a standard 1.5 μg/kg open oral challenge with glucose developed a higher rate of chemical hypoglycemia (78%-89%) (glucose nadirs below 60 mg/dl or hypoglycemia index above 1.0) than typically found in the general population. However, in this initial open study, Am. J. Psychiatry , panic patients did not develop panic attacks, despite other symptomatic evidence of hypoglycemia such as hunger, diaphoresis, tremor, and lightheadedness. These findings suggested that nonspecific perturbations of the autonomic nervous system do not inevitably lead to the subjective experience of panic attacks in panic disorder patients. Recent unpublished findings from another research team which failed to elicit panic attacks in panic disorder patients following insulin-induced hypoglycemia supports our hypothesis.

During the past year we have extended these earlier findings by investigating the effects of blindly administered glucose 1.5μg/kg in panic disorder patients and normal controls. Each subject was randomly given either glucose or an active placebo (saccharin with lemon juice). Neither patients nor controls could distinguish between glucose and the saccharin conditions. Although there was no difference between the two groups following saccharin (active placebo), the panic patients compared to normals developed significantly more symptoms of autonomic arousal following glucose. Of interest, there was no difference on any chemical measure of hypoglycemia between the two groups following glucose. In contrast to our earlier findings, these new results suggest that panic patients may exhibit glucose intolerance, rather than a problem with hypoglycemia, compared to normal controls. Additional studies are indicated to elucidate the neurobiology of the apparent glucose intolerance demonstrated by these new findings with the glucose tolerance test.

8. GH-response to Clonidine

Studies using clonidine to assess noradrenergic function continue to be investigated by the Unit on Anxiety and Affective Disorders. Several lines of evidence, reviewed in detail elsewhere, suggest that the GH-response to clonidine may provide an index of postsynaptic α_2-adrenergic function. Since noradrenergic dysfunction, particularly noradrenergic overactivity, represents one of the major current theories of anxiety, the GH-response to clonidine was studied in 11 nondepressed panic disorder patients compared to depressed patients and normal controls triple-matched for age, sex, and menstrual cycle status. Any matched-triplet containing an individual with baseline GH values greater than 3 ng/ml was excluded, leaving a cohort of seven matched-triplets for analysis. Data were evaluated by analysis of variance (ANOVA). Clonidine-induced GH increases were significantly blunted in the panic-anxious (baseline: $\bar{x}$ = 1.31 ± 0.25 S.E. vs. peak: $\bar{x}$ = 1.53 ± 0.23) and depressed (baseline: 1.80 ± 0.22 vs. peak: 2.19 ± 0.39) patients compared to normal controls (baseline: 2.19 ± 0.24 vs. peak: 9.86 ± 2.10) ($F = 12.26$, $p < .0004$). In addition to indicating a "downregulation" of postsynaptic α_2-adrenergic receptor responsiveness, these data can be added to previous reports suggesting that disturbances in noradrenergic function may be common to both panic and major depressive disorders.

9. Panic Disorder and the Hypothalamic-Pituitary-Adrenal Axis

Several lines of evidence have suggested both similarities and differences between panic and major depressive disorders, suggesting a partial overlap in the neurobiology of these two disorders. As reviewed in Section 5 of this report and elsewhere, similar alterations in noradrenergic function and platelet dihydroergocryptine binding in these two groups led the Unit on Anxiety and Affective Disorders to investigate, in nondepressed panic disorder patients, several neuroendocrine tests (TSH, CRH, and DST) which may be indirectly modulated by the noradrenergic neurotransmitter system. Of interest, these neuroendocrine tests have all been previously reported to be disturbed in melancholic depression.

a. TSH Response to TRH. In collaboration with Drs. P. Roy-Byrne, P. Gold, D. Rubinow, and R. Post, TRH was given to 12 patients with panic disorder (six males and six females) who ranged in age from 23 to 36, with a mean age of 30, and to ten normal volunteers (six females and four males) who ranged in age from 21 to 41 with a mean age of 26.2. After an overnight fast, TRH tests were performed between 8:30 am and 9:30 am by slowly infusing 500 μg of TRH 20 minutes after insertion of an intravenous line and drawing blood samples through the i.v. at 0, 15, 30, and 45 minutes.

Using a criterion of Δ max TSH < 7 uIU/L, four of 12 panic disorder patients had a reduced TSH response to TRH while none of ten controls did (Fishers exact test, $p = .06$). As a group compared with normals, patients had significantly lower Δ max TSH values (8.72 ± 1.53 S.E.M. vs. 15.76 ± 1.50 S.E.M., $p < .001$). Although basal TSH levels were slightly lower in patients than controls, this difference did not account for the lower Δ max TSH values in patients since there was no correlation between basal TSH and Δ max TSH.

Factors most frequently noted to be capable of reducing TSH response to TRH include hypercortisolism, increased hypophyseal dopamine, and somatostatin. Additionally, norepinephrine has been shown in animals to stimulate the release of TRH. This raises the possibility that the noradrenergic hyperactivity postulated to occur in some patients with panic disorder could theoretically cause TRH overdrive and subsequent desensitization of the thyrotrope.

b. ACTH and Cortisol Responses to CRH. In collaboration with Drs. P. Roy-Byrne, P. Gold, D. Rubinow, and R. Post, a CRH test was performed on eight patients with panic disorder (four females and four males). Their mean age was 30. The results were compared with results of CRF tests done under similar conditions at the NIMH in the past year on 27 normal controls and 18 depressed patients. CRH tests were performed at 8:00 pm following a six-hour fast using a dose of 1 μg/kg of ovine CRF. Following i.v. insertion and blood sampling at -15 and -5 minutes, CRH was infused over one minute and blood drawn at 15, 30, 60, 90, 120, 150, and 180 minutes.

Compared with normal controls, panic disorder patients had reduced ACTH response ($p < .01$) and reduced cortisol response ($p < .001$) and ACTH ($p < .05$) levels. These results are similar to those found in depressed patients who, compared with normals, had reduced ACTH responses ($p < .001$), reduced cortisol responses ($p < .02$), and elevated basal cortisol levels ($p < .001$).

The occurrence of reduced ACTH and cortisol responses to CRH in patients with panic disorder resembles the abnormally reduced responses seen in patients with major depressive disorder. In contrast to studies showing normal suppression of cortisol following dexamethasone in patients with panic disorder (see 6c, this report) and both normal baseline cortisol levels and failure of cortisol to rise following panic attacks induced by lactate infusion in the morning, we found impressive elevations of night-time cortisol in our patients. Since these elevations did not occur in either depressed patients or normals, they suggest that panic disorder patients may be either more sensitive to the stress of anticipating an infusion, or just more hypercortisolemic at night.

In light of this possible night-time hypercortisolism, the reduced ACTH responses of these patients (as in depressed patients), may be seen as "appropriate"; i.e., the corticotroph is responding to feedback inhibition by the target gland hormone. In addition to the hypercortisolemia, other factors may have contributed to the reduced ACTH response. Somatostatin, not yet examined in panic disorder patients, is able to reduce the ACTH response to CRF in cell cultures. Norepinephrine added to cell cultures is known to potentiate CRF stimulation of ACTH and to increase levels of ACTH. Thus, alterations in systems modulated by somatostatin and/or norepinephrine may influence in part the apparent reduced ACTH and cortisol responses to CRF in patients with panic disorder.

c. Dexamethasone Suppression Test. In collaboration with Drs. P. Roy-Byrne and L. Bierer, 16 patients with panic disorder (nine women and seven men) ranging in age from 23 to 48 ($\bar{x}$ age = 33 years) and 22 normal volunteers (16 women and six men) ranging in age from 20 to 49 ($\bar{x}$ age = 30

years) were tested with a standard DST. All subjects were given two 500 μg tablets of dexamethasone (1 mg) to take at 11:00 pm and blood samples for cortisol were drawn at 4 pm the following afternoon.

There was no significant difference in the mean post-dexamethasone cortisol values between the patients (2.9 ± 1.3, $\bar{x}$ ± S.E.M.) and the controls (2.2 ± 0.6) ($t = 0.55$, p = NS). Using a criterion cortisol value of greater than 5 μg/dl to indicate nonsuppression, there was no significant difference between the proportion of patients (4/16 or 25%) and normals (3/22 or 14%) with "abnormal" tests (Fischers exact test, $p = 0.44$). The 95% confidence interval for 4:00 pm post-dexamethasone cortisol value was calculated using the normal control data. Using the upper limit of this confidence interval ($\bar{x}$ ± S.D.) as the criterion cortisol value (i.e., 7.1 μg/dl) only 1/16 or 6% of patients actually showed "abnormal" post-dexamethasone cortisol values compared with 1/22 or 4% of controls (Fischers exact test, $p = 0.48$). Although the number of subjects in the patient sample was too small to compare the clinical characteristics of suppressors to non-suppressors statistically, it should be noted that the one patient with the most abnormal cortisol value (20.6 μg/dl), as well as the other three with cortisol values above 5 μg/dl, were no more depressed or anxious or likely to have had panic attacks in the recent week than the patients who suppressed normally. In contrast, the one patient who had a panic attack earlier on the day of dexamethasone administration suppressed normally.

Our results are consistent with three earlier reports suggesting that panic disorder patients do not have an abnormal incidence of cortisol nonsuppression following dexamethasone. One recent report, however, found that seven of 15 (45%) panic disorder patients with secondary depression showed cortisol nonsuppression following dexamethasone. Since our patients were not depressed, it remains to be seen if panic disorder patients suffering a "secondary" depression have a higher incidence of abnormal DSTs.

II. Proposed Course of Study

A. Animal Research

During the past two years, the Unit on Anxiety and Affective Disorders has established a viable colony of "normal" and "nervous" pure-bred pointer dogs. These dogs offer the advantage of investigating both "normal" behavior and "spontaneously-occurring" (rather than laboratory-conditioned) fear behaviors. The "nervous" line may be particularly useful in the study of several behaviors and characteristics relevant to human psychopathology, including genetically-transmitted inheritance with phenotypic expression of "nervous" behaviors at eight to 12 months of age. This delayed manifestation of pathology in dogs parallels in a similar, temporal fashion, the emergence of agoraphobia in humans during adolescence and early adulthood.

While the colony was being established the development of observation chambers with one-way mirrors and rating scales were developed in collaboration with Dr. S. Weiss. In collaboration with Dr. E. Klein, careful and precise techniques for the surgical removal of the whole brain under general anesthesia have been developed and already performed in approximately six dogs from each

line, "nervous" and "normal" pointer dogs. A brain mold for the purebred pointer dog has been developed as well during the past six months and will allow the Unit to prepare regional brain tissue immediately after surgical removal for later α_2-adrenergic, benzodiazepine, imipramine, opiate, and other binding studies. In collaboration with Drs. E. Klein and R. Lenox, brain yohimbine and clonidine binding will first be investigated. These and other ligands will be studied as well in other body tissues, including the pituitary, heart, adrenal gland, and kidney organs which have all been implicated in the mediation of stress response patterns in nonhuman and human primates.

In addition to these brain and other tissue studies, research investigating the behavioral psychopharmacology of these dogs will be pursued. Initial studies will focus on behaviors modulated by benzodiazepine, GABA, and adenosine receptor systems. In collaboration with Drs. S. Weiss and R. Post, we have already investigated the effects of diazepam and Ro 15-1788 in approximately 20 pointer dogs. Evidence suggests that although Ro 15-1788 reversed diazepam-induced hind leg ataxia, Ro 15-1788 did not reduce most of the spontaneous abnormal behaviors of the nervous pointer dog. These preliminary data suggest that most abnormal behaviors in this animal model of "anxiety" are unlikely to be mediated by an endogenously-produced anxiogenic ligand whose main effects are produced by binding to the benzodiazepine receptor. Future studies will focus on the elucidation of the neurobiological underpinnings of these familial abnormal nervous behaviors in the purebred pointer dog.

B. Human Research

Human research conduced by the Unit on Anxiety and Affective Disorders has demonstrated a blunting of the clonidine-induced growth hormone (GH) response in panic and depressed patients compared to age- and sex-matched controls. These findings suggest decreased postsynaptic noradrenergic function in these disorders. Other lines of evidence reviewed in last year's annual report (Z01 MH 00071-04 BP) also suggest a similarity in noradrenergic dysfunction in these two disorders. As a result, the Unit will continue to investigate the noradrenergic system in relation to psychopathological syndromes, mechanisms of drug action, and treatment response. Thus, we plan to continue the yohimbine challenge study to further assess noradrenergic function in patients with pathological anxiety and depression. The behavioral and biochemical effects of alprazolam on yohimbine-induced anxiety will be investigated in panic patients.

We also intend to expand our research with caffeine. Further delineation of the clinical response to caffeine is indicated because caffeine consumption is correlated with symptoms of generalized anxiety in patients with panic attacks, but not in normal volunteers. Caffeine derivatives also activate noradrenergic activity in animals when iontophoretically applied to the locus coeruleus. Furthermore, caffeine has been shown to antagonize the biochemical and pharmacological effects of benzodiazepines, and alprazolam, a triazolabenzodiazepine, blocks the typical time course of caffeine-induced arousal, panic attacks and generalized anxiety in humans (see sections b and d, this report). Other lines of evidence suggest a major role for adenosine-regulated systems in the mediation of caffeine's psychostimulant properties. All three of these

systems have been independently implicated in the neurobiology of anxiety and stress. Moreover, caffeine was found by our Unit to significantly elevate measures of the stress-related hormone, cortisol, and induce cortisol escape from dexamethasone suppression. Thus, we intend to extend and expand our ongoing research with caffeine by investigating the behavioral, physiological, neuroendocrine, and biochemical effects of this and other methylxanthines in both animals and humans.

Studies of the clinical efficacy of alprazolam, clonidine, carbamazepine, imipramine, and propranolol in panic and phobic patients will be continued. Our preliminary research with clonidine in depressed and anxious patients and normal volunteers is encouraging and suggests that clonidine might be especially useful in patients who experience context-dependent anxiety (e.g., anticipatory anxiety) as well as some patients with "spontaneous" panic attacks. Studies with verapamil, a calcium channel blocker, will also be initiated. These clinical trials, in conjunction with concomitant measurements of the neurotransmitter effects, should enhance our understanding of alterations in neurotransmitter pathways associated with pathological anxiety and its amelioration with appropriate psychopharmacotherapies.

III. Significance to Biomedical Research and the Program of the Institute

Several epidemiological surveys have suggested that pathological degrees of anxiety may adversely influence a large segment of our population. Agoraphobia, an anxiety syndrome associated with "spontaneous" panic attacks, results each year in the impairment of individuals previously well-functioning and productive. Pathological anxiety has been recently found to be the second most prevalent mental health problem in this country. The role of anxiety and stress in coronary heart disease and other medical illnesses has been suggested by a number of studies. Moreover, emerging epidemiological and familial data suggest that a subgroup of patients with major depressive illness plus panic attacks may represent an important and distinct subtype of major affective illness. We intend to investigate biological correlates in the plasma and cerebrospinal fluid of this subtype, who may be a greater risk for alcoholism and suicide, compared to patients with major depressive illness without panic attacks. An improved understanding of the clinical and biological aspects of both normal and pathological anxiety is thus critically needed. It is hoped that the developing battery of clinical and biological tests in patients with anxiety and related mood disorders will ultimately provide a clinical and biological profile of these illnesses and lead to more refined subcategorizations, as well as to more selective and efficacious treatment approaches.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00452-10 BP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neuroendocrine Studies of Major Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.4	3.0	2.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current neuroendocrine studies focus on elucidating normal physiology and pathophysiological mechanisms in controls and patients with major psychiatric and neuroendocrine diseases. Our studies with corticotropin releasing hormone (CRH) suggest that this peptide functions as a physiologically relevant stimulus to the pituitary-adrenal axis in man. Hence, eight 1 μg/kg pulses of human CRH (hCRH) given over 24 hours in a temporal sequence designed to mimic naturally occurring ACTH pulses, restored the normal pulse frequency and amplitude of ACTH and cortisol secretion in patients with hypothalamic CRH deficiency. This study also showed that the ACTH response to hCRH was enhanced in the early morning, suggesting that the pituitary corticotroph cell shows a circadian rhythm in its response to CRH. This idea is supported by our finding in volunteers that the early morning cortisol surge is associated with an increase in both the pulse frequency and the amplitude of ACTH secretory episodes. Our previous suggestion that hypercortisolism in depression reflects hypersecretion of CRH is supported by data that abnormally high post-dexamethasone plasma ACTH levels in depression are negatively correlated with CSF CRH. In addition, our previous suggestion that the hypothalamic CRH neuron in Cushing's disease is normally suppressed by long-standing hypercortisolism is supported by data that CSF CRH is markedly reduced in this disorder. In an enlarged series of depressed and Cushing's disease patients, the CRH stimulation test remains helpful in their differential diagnosis. In patients with anorexia nervosa, responses to CRH stimulation and an elevation in CSF CRH suggest that the hypercortisolism in this disorder reflects the hypersecretion of endogenous CRH. A second study in anorexia nervosa utilizing growth hormone-releasing hormone shows that the hypersecretion of GH in this disorder reflects a somatomedin deficiency, secondary either to hypercortisolism or chronic inanition. Other findings include decreased CSF CRH and ACTH in Alzheimer's disease unassociated with hypofunction of the pituitary-adrenal axis.

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I. Project Description

A. Objectives

The fundamental strategy of the Section on Clinical Neuroendocrinology has been to extend current concepts regarding the physiology of neuroendocrine regulation in animal models and healthy volunteers, and to apply this understanding in an effort to unravel the pathophysiology of neuroendocrine disturbances in a variety of patient populations. These clinical populations span multiple disciplines including patients with several major psychiatric diagnoses (e.g., affective illness, anorexia nervosa, panic anxiety disorder, schizophrenia, alcoholism), as well as a range of major neuroendocrine abnormalities (e.g., Cushing's disease, ectopic ACTH secretion, ACTH-independent hypercortisolism, primary and secondary adrenal insufficiency, Nelson's syndrome, and diabetes insipidus).

Our work on neuroendocrine regulation in patients with major psychiatric illness reflects a rapidly enlarging body of literature which suggests an intimate linkage between neurohormonal functional activity and major components of the symptom complexes of illnesses such as depression and anorexia nervosa. For instance, several aspects of the syndromes of primary affective disorder and anorexia nervosa suggest hypothalamic dysfunction. For instance, patients with depression often manifest disturbances in appetite, libido, reproductive function (i.e., amenorrhea), water metabolism, and in the temporal organization of a variety of phenomena whose circadian periodicity is thought to be governed by hypothalamic pacemakers. Patients with anorexia nervosa show not only profound alterations in eating behavior, but also marked changes in hypothalamic-pituitary function, including abnormalities in vasopressin secretion, growth hormone regulation, gonadotropin secretion, and in plasma levels and production of cortisol. In addition to efforts to explore the mechanism of these symptoms in psychiatric populations, our interest in neuroendocrine systems also relates to the fact that the monoaminergic neurotransmitters long thought to play a dominant role in major psychiatric illness modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones; thus, examination of pituitary hormones in plasma can shed light on the functional activity of biogenic amine systems. Moreover, the hypothalamic hormones themselves have been shown to be widely distributed within brain, to exert specific receptor-mediated biological actions, and to influence the functional activity of brain neurotransmitter systems. Several hypothalamic hormones have also been shown to have profound effects on coordinating complex behaviors and physiological processes of relevance to adaptation and the maintenance of internal homeostasis.

Our interest in neuroendocrine regulation in patients with medical illnesses such as Cushing's disease reflects our commitment to the clinical study of neuroendocrine regulation relevant to health and disease. We have focused our work on patients with abnormalities of the hypothalamic-pituitary-adrenal (HPA) function, since it is this axis which is most consistently disturbed in a variety of psychiatric illnesses and because some major neuroendocrine disturbances in such patients are difficult to clinically distinguish from major psychiatric illness. In particular, one of our goals is to comprehensively compare and contrast the pathophysiology of the hypercortisolism in depression and Cushing's disease and to develop a means of assisting in their often difficult differential diagnosis. We also hope to explore possible mechanisms for elucidating the differential diagnosis between Cushing's disease and ectopic ACTH secretion, and among various

causes of adrenal insufficiency. Although our work in both psychiatric and medical populations focuses on HPA regulation, we also attempt to integrate data concerning the regulation of other neurohormones in these patients, including arginine vasopressin, oxytocin, somatostatin, the endogenous opiates, and growth hormone-releasing hormone.

In our clinical studies, several neuroendocrine strategies have been routinely utilized: 1) direct measurement in the CSF and in the plasma of behaviorally active peptides during the basal state and/or following stimulation according to verified stimulation paradigms; 2) administration of hypothalamic releasing factors to test responses of the pituitary axis and to elucidate patterns of endogenous monoaminergic disturbance and neuroendocrine dysfunction; 3) elucidation of the effect of psychoactive agents on hypothalamic-pituitary axis function and on levels of behaviorally active peptides; 4) assessment of the temporal organization of neuroendocrine function;

As an adjunct to our clinical studies, we maintain an active preclinical study program to explore neuroendocrine regulation in non-human primates and other experimental animals. In our primate model, we have developed a new strategy which utilizes surgical placement of i.c.v. and i.v. cannulae in primates, which allows sampling without restraint. We have also established models in the sheep to study neuroendocrine regulation. Our preclinical and clinical studies are supported by an active laboratory which has developed assays for a wide variety of peptide hormones and for several modified synthetic steroids such as the glucocorticoid antagonist RU-486. We also have the capacity to perform tissue culture studies to conduct a range of studies concerning the functional activity and regulation of neurohormonal systems.

1. Studies of Behaviorally Active CNS Peptides:

a. Corticotropin-Releasing Hormone (CRH)

CRH is a 41-amino acid peptide which was first isolated from ovine hypothalami by Vale et al. in 1981. This peptide showed greater *in vivo* and *in vitro* corticotropin releasing potency than any previously identified synthetic or endogenous peptide. Shortly thereafter, Schally et al. described the sequence of porcine CRH, and Rivier et al. that of rat CRH (rCRH). Finally, Neuma et al. sequenced the genes of both ovine CRH (oCRH) and human CRH (hCRH) and deduced the amino acid sequence of the corresponding peptides. Surprisingly, both the rCRH and the hCRH appeared to be chemically identical. Moreover, oCRH and h/r CRH are also structurally similar, each containing 41 amino acids and showing 83% homology.

The sequencing and subsequent synthesis of CRH has greatly enhanced the capacity for clinical neuroendocrinologists to explore the hypothalamic-pituitary components of Cushing's disease and adrenal insufficiency. CRH is also of importance to clinical psychoneuroendocrinologists, since its discovery occurred in the context of two other discoveries that have heightened their interest in the HPA axis and its regulation. First, many patients with depression, anorexia nervosa, alcoholism, bulimia, obsessive-compulsive neurosis, and dementia have a hyperactive pituitary-adrenal axis. In fact, the hypercortisolism seen in depressive illness and alcoholism can be so severe that it is difficult to distinguish them from Cushing's disease; hence, each of these psychiatric entities has been referred to colloquially by some endocrinologists as pseudo-Cushing's states. Second, it has

been shown that ACTH is secreted synchronously with beta-endorphin, one of the principal endogenous opioid peptides, and that both hormones are contained within the sequence of a common precursor molecule, pro-opiomelanocortin (POMC). CRH is the principal central signal for the cleavage of pituitary POMC into biologically active peptides.

CRH is also of interest to psychiatrists and neurobiologists for reasons other than its putative role in regulating the pituitary-adrenal axis. Hence, CRH is synthesized not only by the hypothalamus with transport by hypophyseal portal blood, but, like other hypothalamic hormones such as TRH and somatostatin, it is either distributed and/or synthesized beyond the boundaries of the hypothalamus in many brain regions, and like these peptides, seems to play a role in coordinating complex behavioral and/or physiological processes. Specifically, it has been shown that there are extensive hypothalamic aggregations of CRH cell bodies and terminal fields in the limbic system, cortex, and in close association with the central autonomic system in the locus coeruleus. This distribution of CRH within and beyond the hypothalamus provides an anatomical context for the observation that CRH can simultaneously activate and coordinate metabolic, circulatory, and behavioral responses that are adaptive in stressful situations. Hence, in the rat, intracerebroventricular (ICV) administration of CRH leads not only to activation of the HPA axis, but also to activation of the sympathetic nervous system and to several behavioral changes characteristic of the stress response, including decreased feeding and sexual behavior, assumption of a freeze posture in a foreign environment, and increased exploration in familiar surroundings. In addition, in collaboration with Drs. Weiss and Post, is has been shown that CRH given ICV to the rat causes a marked increase in hostility and induces limbic seizures that show crosssensitization with electrically-kindled seizures.

Given CRH's significant role in HPA regulation and its intriguing effects on CNS function, we embarked on a series of clinical studies with CRH in both normal volunteers and in patients with major psychiatric disorders whose illnesses are at times characterized by hypercortisolism (e.g., primary affective disorder, anorexia nervosa, panic anxiety disorder, and schizophrenia). In addition, we studied patients with endocrine disturbances characterized by abnormal HPA function, including subjects with Cushing's disease, ectopic ACTH secretion, primary and secondary adrenal insufficiency, and Nelson's syndrome. In volunteers, we hoped to examine the physiological relevance of CRH to pituitary-adrenal function in man as well as to explore the differential biological effects and pharmacokinetics of oCRH and hCRH under varying conditions. In our patient populations, we asked the following questions: (1) Can CRH help determine whether the hypocortisolism in depression reflects an alteration in the setpoint for feedback inhibition of cortisol on ACTH secretion at the pituitary locus, versus the possibility of an alteration in the secretion of endogenous CRH? (2) Can CRH help in the differential diagnosis of the various hypercortisolemic psychiatric syndromes? (3) Can CRH help determine whether depression and Cushing's disease lie on a common pathophysiological continuum or represent distinct abnormalities of the HPA axis? (4) Can CRH help establish the differential diagnosis of disturbances in HPA function, which can be difficult to distinguish from one another, such as depression from Cushing's disease, ectopic ACTH production from Cushing's disease, or hypothalamic from pituitary-adrenal insufficiency?

Studies of CRH in Normal Volunteers

To address the question concerning the physiological relevance of CRH to pituitary regulation in man we first studied normal controls to compare naturalistically occurring ACTH pulses to those induced by hCRH administration. We noted that a comparison of spontaneous endogenous ACTH and cortisol surges (isolated during normative circadian studies) is identical to those induced by an intravenous bolus of 1 μg/kg of hCRH. This finding, that hCRH-induced plasma ACTH and cortisol pulses mimicked the spontaneously occurring secretory episodes of these hormones, provided the first compelling evidence that CRH is indeed of physiological relevance to the pituitary-adrenal axis in man and led to an additional experiment designed to further explore the relationship between pulsatile CRH administration and the patterns of ACTH and cortisol secretion in the basal state. In this study, we asked the following question. Could hCRH restore the function of the pituitary-adrenal axis seen in patients with CRH deficiency; i.e., secondary adrenal insufficiency due to corticotropin-sparing (suprapituitary) lesions?

The patients included in this study had secondary adrenal insufficiency determined by a standard 48-hour ACTH stimulation test. During the three hour evening oCRH test, the plasma cortisol response of these patients was diminished while the plasma ACTH response was normal or exaggerated and showed a delayed pattern suggestive of a hypothalamic lesion. hCRH was given as a 1 μg/kg bolus eight times during the 24-hour period. The timing of each pulse of hCRH was chosen to correspond to the expected times of ACTH pulsation under naturalistic conditions. Hence, five of the eight pulses were given in the early morning hours, at the time of the expected cortisol surge. In light of data on other releasing factors, showing that the pulsatile hypothalamic hormone secretion into the hypophyseal portal system seems to occur either every 90 minutes or in multiples of 90 minutes, we chose this interval for the present study. Accordingly, the five early morning pulses of hCRH were given concurrently every 90 minutes from 2:30 AM to 8:00 AM and the remaining three pulses were given at various times after 8:00 AM at intervals which were multiples of one-and-one-half hours (i.e., every three to six hours).

The results of this study show that pulsatile administration of hCRH in the manner described above reproduces the normal amplitude and circadian variation of cortisol secretion in patients with hypothalamic-CRH deficiency. Moreover, the administration of eight pulses of synthetic hCRH, as described above, normalized 24-hour urinary 17-hydroxysteroid and urinary free cortisol secretion in these patients. Parenthetically, we have previously noted that a continuous infusion of oCRH in normal volunteers for 24 hours produces a pattern of cortisol secretion that includes preservation of a circadian rhythm, though the amplitude is blunted compared to the naturalistic rhythm of that induced by the hCRH pulses. Thus, although basal circadian cortisol secretion may be dependent on endogenous CRH secretion, the typical circadian pattern of pituitary-adrenal function may also involve a component of a circadian rhythm in the pituitary corticotroph cell in response to CRH itself. The idea that the corticotroph cell shows a circadian rhythm in response to CRH is further suggest by our pulsatile hCRH administration data in patients with hypothalamic CRH deficiency (in which the responses to the early morning pulses are greater than the responses to the daytime pulses), and by our own delineation of the naturalistic pattern of circadian ACTH secretion in normal volunteers. This latter study showed that the early morning cortisol surge is associated with an increase in both the pulse frequency and amplitude of ACTH secretion.

Relevance of CRH to Stress-Induced ACTH Secretion

In addition to studies of the relevance of CRH to basal and circadian ACTH and cortisol secretion, we have also attempted to assess the relationship of CRH to pituitary-adrenal function during stress. To accomplish this task, we have attempted to see whether the ACTH responses to frequent pulses of hCRH given at 30-90 minute intervals from 1800-2000 h would produce an ACTH secretory pattern resembling that seen during the standard insulin tolerance test. We noted that the ACTH and cortisol responses to repeated pulses of hCRH were less than those seen during the insulin tolerance test. In light of this work and data from experimental animals in which neutralization of endogenous CRH in rats by administration of anti-CRH antibodies abolished only 75% of the ACTH responses to ether stress, we conclude that factors other than CRH play at least some role in producing the ACTH responses seen during stress. Putative factors that may underlie these extra-CRH influences on stress-induced ACTH secretion include the catecholamines and AVP, both of which are known to increase during hypoglycemia and other forms of stress. In support of the possible role of vasopressin is our recent data obtained in normal volunteers, in collaboration with Dr. Rittmaster, which shows that osmotically-induced vasopressin secretion markedly potentiates CRH-induced ACTH secretion. We doubt that oxytocin participates in CRH-induced ACTH secretion, despite a suggestion from *in vitro* studies, where it has been shown to have synergy with CRH in causing ACTH secretion. In collaboration with Dr. Nieman, we have observed no synergy of oxytocin with hCRH in eliciting ACTH secretion in man.

Development of a Clinically Applicable CRH Stimulation Paradigm: Characterization of the Dose-Response Relationships and Pharmacokinetics of oCRH and hCRH and Assessment of their Effects at Different Times of Day

To ascertain the clinical applications of CRH, we initiated a series of studies in volunteers to assess the following questions: (1) which peptide (oCRH or hCRH) might be best to use in acute challenges of the pituitary-adrenal axis; (2) what dose should be administered in these studies and for how long should hormonal responses be sampled; (3) what time of day would be best suited for the performance of dynamic stimulation of the human pituitary-adrenal axis? To assess these questions, we conducted pharmacokinetic and dose-response studies with both oCRH and hCRH. Some of these studies were performed at different times of day to correspond to periods when the HPA axis is normally quiescent or more active.

The first dose-response study with oCRH in primates was performed by our group in cynamolgus macaques. Corresponding studies in man, yielding similar results, were performed by Grossman et al. and Orth et al. These studies show that the lowest maximal stimulatory dose for cortisol secretion was 1 μg/kg; moreover, this dose produced clearcut plasma cortisol and ACTH secretion in all volunteers and experimental animals without detectable adverse effects. Of particular interest was the fact that the ACTH and cortisol responses to oCRH were prolonged, remaining clearly elevated at the end of the three-hour sampling period.

In similar dose-response studies with hCRH in non-human primates and man, we also noted a dose-dependent increase of plasma ACTH and cortisol concentrations with greater doses of hCRH. Peak plasma ACTH and cortisol responses to hCRH were slightly lower than those achieved by oCRH. Moreover, the ACTH and cortisol responses to hCRH were of much shorter duration than those with oCRH. Accordingly, comparisons of the integrated secretory responses of both ACTH and cortisol fol-

lowing hCRH administration indicate that oCRH is at least five-times more potent than hCRH. This difference is mainly due to the longer-lasting effect of oCRH upon ACTH and cortisol secretion.

These longer-lasting effects of oCRH on ACTH and cortisol secretion presumably can be accounted for on the basis of the differential pharmacokinetic properties of oCRH and hCRH in human plasma. Hence, a comparison of the metabolic clearance rate of these two peptides in human volunteers reveals that hCRH is cleared from plasma much more rapidly than oCRH. On the basis of the relatively slower clearance and more prolonged biological effects of oCRH, we elected to use this peptide for characterizing the functional integrity of the pituitary corticotroph in our clinical populations. We reasoned that the extra information provided by a pulse of oCRH might provide the kind of additional information that could be helpful in exploring the pathophysiology of hypothalamic-pituitary-adrenal activity in different patient subgroups. Moreover, we felt that this additional information could also be helpful in determining subtle differences in responses among patient subgroups and, hence, more helpful in establishing differential diagnoses among clinical entities. On the other hand, as illustrated by the studies in patients with hypothalamic CRH deficiency, the much more rapidly cleared hCRH seems far more suitable than oCRH for studies of pulsatile ACTH secretion.

An additional factor of relevance to the establishment of a clinically applicable CRH stimulation test is the determination of an optimal time of day for administration of the peptide to patient populations. To explore this question, in collaboration with Dr. Schulte et al, we administered 1 μg/kg bolus of oCRH at 9 AM, near the time of day when the axis is most active, and at 8 PM, when the axis is normally dormant. We found that, owing to the lower baseline ACTH and cortisol levels seen in the evening, the net integrated ACTH and cortisol responses to oCRH are greater at this time. Hence, we decided that our CRH stimulation test would consist of the 1 μg/kg bolus of oCRH given at the 8 PM time. The hormonal responses to this stimulation paradigm indicating effects on ACTH and cortisol, but not GH, PRL, LH, FSH, AVP, PRA, or plasma insulin, show that oCRH is a potent, specific stimulus to the pituitary-adrenal axis in man.

Clinical Studies with oCRH in Patients with Major Psychiatric Disorders

We have studied patients with CRH who carry several psychiatric diagnoses. We were particularly interested in subjects whose illness is characteristically associated with hypercortisolism at some time during its course, including primary affective disorder, anorexia nervosa, and panic anxiety disorder. We also studied a group of psychotic schizophrenic subjects, despite the fact that these individuals are classically thought not to show hypercortisolism.

Our studies of the neuroendocrine responses to oCRH in psychiatric patients are most extensive in individuals with primary affective disorder. To date, 45 drug-free hospitalized patients with Research Diagnostic Criteria-validated affective illness have received oCRH at 2000h in a fashion identical to that described for normal volunteers. Twenty-eight patients were studied while depressed, 11 while improved, and six while manic. All subjects were in good physical health and showed no evidence of renal, hepatic, or thyroid disease.

Our data indicate that the group of depressed patients show evening basal hypercortisolism despite normal basal ACTH levels. Associated with this hypercortisolism was a marked attenuation in the ACTH response to exogenous CRH. This finding suggests that the pituitary corticotroph cell in depression is appropriately restrained by the negative feedback effects of the long-standing hypercortisolism in depression. This relationship between the basal hypercortisolism and the integrated ACTH response to CRH in exogenous depression is supported by a significant negative correlation between the basal cortisol levels and the ACTH responses to CRH in both the depressed patients and the overall groups of depressed, manic, and recovered subjects with primary affective disorder.

In light of the apparently normal corticotroph cell function in depressed patients, we advanced the hypothesis that hypercortisolism in depression represents a defect at or above the hypothalamus which results in the hypersecretion of endogenous CRH. To test this hypothesis, we, in collaboration with Dr. Schulte, attempted to replicate, in normal controls, a situation in which the pituitary corticotroph cell is exposed to excessive corticotropin releasing hormone. In order to accomplish this, we administered a continuous infusion of oCRH for 24 hours and evaluated the ACTH and cortisol responses. Of interest is the fact that the circadian rhythm of cortisol is preserved despite the continuous administration of oCRH, suggesting that the pituitary corticotroph cell shows a diurnal sensitivity to exogenous CRH. The preservation of this circadian rhythm is also of importance, in light of the fact that the circadian rhythm of cortisol is also preserved in a majority of depressed patients who have been studied. Of additional interest is the fact that the mean amplitude of cortisol secretion during continuous CRH infusion is elevated about 40-50%, and that the urinary free cortisol secretion during CRH infusion averaged 150-200 μg/day. Hence, the amplitude of plasma cortisol during the 24-hour period and the magnitude of urinary free cortisol hypersecretion is very similar during conditions of continuous administration of oCRH to controls and in the endogenously depressed state. We conclude, therefore, that a continuous CRH infusion to normal volunteers reproduces the pattern and magnitude of hypercortisolism typically associated with depression.

A further inspection of the ACTH and cortisol responses to CRH in depression showed that the cortisol response seems robust despite attenuated ACTH response in normal controls. This suggests that the adrenal cortex is hyper-responsive to the ACTH released by depressed patients during CRH stimulation. Mathematically, this is represented by the fact that the cortisol to ACTH ratio in depressed patients is significantly greater than in control subjects. This finding is compatible with the well-described phenomenon of progressive functional and anatomical hypertrophy of the adrenal cortex occurring during either experimentally induced stress or during the course of chronic and repeated hyperstimulation of the adrenal cortex by ACTH in man. The suggestion noted here of adrenal hyper-responsiveness to ACTH in depression is compatible with the study of Amsterdam et al., which showed that chronically depressed patients manifest greater cortisol responses to a bolus of exogenous ACTH than normal subjects.

Although our depressed patients were hypercortisolemic, it is noteworthy that basal ACTH levels remained in the normal range. This "normal" plasma ACTH in depression most likely reflects a normal corticotroph cell caught in the balance between forces; i.e., negative feedback exerted by a hyperactive adrenal cortex from below and a predominating excess of CRH drive from above. Hence, the corticotroph

cell, though restrained by the negative feedback to secrete at a rate that produces ACTH levels in the normal range, is nevertheless sufficiently driven by CRH to promote excessive cortisol secretion by hyperplastic adrenals. Presumably, depressed patients would have shown elevated levels of ACTH in the beginning of their depressive illness. Parenthetically, this model is supported by preliminary data from our group which shows that depressed patients show an increased number of low amplitude ACTH pulses/24 hours associated with a similar number of relatively high amplitude cortisol pulses.

We have also explored the pathophysiology of hypercortisolism and other major psychiatric disorders, including anorexia nervosa. The group of underweight anorectic patients we studied showed hypercortisolism that was even more severe than that seen in depressed patients. However, like the depressed patients, these underweight anorectics manifest a markedly attenuated ACTH response to exogenous CRH. This finding strongly suggests that, as in the depressed patients, the pituitary corticotroph cell in anorexia nervosa is appropriately restrained by the negative feedback effects of hypercortisolism, and also suggests that anorectics, like depressed patients, show a defect in the secretion of CRH. In support of this hypothesis is our finding that underweight patients with anorexia nervosa show a significant increase in the level of CRH in the CSF. When the anorectic subjects who were underweight were restudied at yet another phase, after their weight had stabilized at 100% of ideal body weight for one month or longer, the basal hypercortisolism had resolved, suggesting normalization of the central defect which resulted in the hypersecretion of endogenous CRH. This postulate is supported by the finding that the normalization of pituitary-adrenal function is associated with normalization of the level of CSF CRH. However, despite normalization of this central defect, these normal weight anorectics continued to show a marked attenuation of ACTH response to CRH. Although we cannot account for this finding, it may represent the persistence of a functionally hypertrophied adrenal cortex into this phase of short-term recovery. On the other hand, normal weight bulimic subjects studied for ten days after a voluntary abstinence from binging and vomiting, and a group of anorexia nervosa subjects who had maintained normal body weight for at least six months, showed normal basal ACTH and cortisol values and their responses to exogenous CRH. From these studies, we conclude that the basic pathophysiology of hypercortisolism in anorexia nervosa is similar to that seen in the depressed phase of primary affective disorder and that subtle defects in HPA function persist in patients with anorexia nervosa despite the return to eucortisolism after the short-term correction of the weight loss. Parenthetically, the level of CSF CRH in anorectic patients was positively correlated with depression ratings, further suggesting that depression and anorexia nervosa lie on a pathophysiological continuum.

We have also studied, in collaboration with Dr. P.P. Roy-Byrne, another psychiatric population that has been shown in some studies to manifest hypercortisolism. This group included subjects diagnosed as having panic-anxiety disorder. These panic-anxious subjects, like the depressed patients and the underweight anorectics, manifested a blunted ACTH response to exogenous CRH in association with significant basal hypercortisolism. Hence, the group of hypercortisolemic psychiatric patients, regardless of diagnosis, show a similar response to exogenous CRH, indicative of a common pathophysiological mechanism underlying the hypercortisolism in these disorders.

In contrast to the three hypercortisolemic psychiatric subgroups who showed the blunted ACTH responses to CRH, a group of severely psychotic schizophrenic patients was studied in collaboration with Dr. A. Roy. These patients showed normal basal cortisol values and normal ACTH responses to exogenous CRH. These data are compatible with previous studies which show that a smaller percentage of patients with schizophrenia than those with affective disturbance manifest overt basal hypercortisolism or fail to suppress their cortisol levels after dexamethasone. When eight of these drug-free schizophrenic patients were re-studied after treatment with fluphenazine, the ACTH and cortisol response to CRH were similar to those seen during the drug-free state. In light of this eucortsolism and the normal ACTH responses to CRH in these psychotic schizophrenic patients, it is intriguing to speculate that perhaps there is some defect in the transmission of the actual experience of distress and danger that these subjects clearly feel to the hypothalamic neurons which translate such anxiety into activation of the CRH neuron. Further work on exploring the perturbability of the CRH neuron in schizophrenic subjects following other stresses would seem warranted to further test this hypothesis.

Whether CRH plays a role in any human disease apart from the rare cases of Addison's disease secondary to CRH deficiency remains to be established. However, we have previously noted that its possible involvement in depression is intriguing in light of the following four sets of findings taken from the disciplines of developmental physiology, clinical psychiatry, and neurophysiology. 1. Laboratory animals subjected to maternal deprivation during the neonatal period show significant hyperactivity of the HPA axis during stress throughout adult life. Hence, such animals presumably show a permanent change in the responsivity of their CRH neuron. 2. Individuals who are depression-prone are thought to show greater than usual incidence of early noxious stress or maternal deprivation. Clinical experience shows that such a history seems to produce a tendency to repetitively relive the intense anxiety and dysphoria associated with this early deprivation throughout adult life whenever a significant frustration or important loss occurs. Thus, such individuals also seem prone to a hyper-responsivity of their CRH neuron intermittently throughout life. 3. CRH given ICV to experimental animals not only stimulates the HPA axis, but also activates the locus coeruleus, produces decreased eating and sexual behavior, and causes significant changes in activity. 4. CRH has been reported to induce limbic seizures which cross-sensitize with electrically kindled seizures. These findings, taken together, suggest that a CRH model of depression could help integrate dynamic formulations which take into account early losses and subsequent internal and external stress as factors which can predispose to or precipitate major depression, and the observations that depressed subjects often show hypercortisolism, significant anxiety, anorexia, diminished libido, hypo- or hyperactivity, and respond at times to limbic anticonvulsants. That changes in CRH may be related to depressive symptomatology is also supported by empirical observations that depression is perhaps the only major symptom represented in a substantial number of patients with each of the various psychiatric disorders characterized during their course by sustained or episodic hypercortisolism. Hence, it is not uncommon that patients with anorexia nervosa and panic-anxiety disorders show both depressive features as well as family histories of depressive illness, suggesting the idea that the hypercortisolism in psychiatric disorders may be part of a depressive spectrum disorder with abnormalities of endogenous CRH secretion as a common denominator.

The CRH Stimulation Test: Implications for the Diagnosis and Pathophysiology of Hypercortisolism in Depression and Cushing's Disease

The hypercortisolism of depression can be of sufficient magnitude that it has been termed a pseudo-Cushing's state. Conversely, patients with Cushing's disease often show signs of clinical depression. Although there has been controversy over the years concerning the etiology of the hypercortisolism associated with affective illness and Cushing's disease, the overlap in the clinical and biochemical manifestations of these illnesses has prompted some to suggest that they share common pathophysiological features. Of clinical significance is the fact that patients with primary depression who may be hirsute or obese and who manifest high plasma and urinary free cortisol levels can be impossible to distinguish from patients with mild or early Cushing's disease. Depression can often be the first manifestation of Cushing's disease, preceding the physical stigmata such as the buffalo hump or purple striae by months or even years.

Data in patients with Cushing's disease show that despite profound basal hypercortisolism, these patients show a marked hyper-responsiveness of the pituitary corticotroph cell to exogenous CRH. Thus, in contrast to patients with depression who show a pituitary corticotroph cell normally responsive to the negative feedback effects of glucocorticoids, patients with Cushing's disease manifest a pituitary corticotroph cell which is grossly unresponsive to cortisol negative feedback effects. Our data also suggest that the differences in pituitary corticotroph cell function between depressed and Cushing's disease patients seem accompanied by differences in hypothalamic CRH neuron function. Specifically, we have shown that nine of our patients with Cushing's disease, whom we studied one week after selective transsphenoidal adenectomy (at a time when basal ACTH and cortisol were uniformly undetectable), showed normal or nearly normal plasma ACTH responses to exogenous CRH. We surmise that the adrenal insufficiency in each of these postoperative patients reflects hypofunction of corticotropin releasing factor neurons which had been physiologically suppressed by exposure to the negative feedback of their long-standing hypercortisolism. This in in contrast to the hypothalamic defect we propose for depression. Such differences in hypothalamic function between depressed patients and patients with Cushing's disease is also supported by our finding that depressed patients have CSF CRH levels several-fold higher than patients with Cushing's disease, whose levels are significantly reduced compared to normal controls.

The differential pathophysiology of hypercortisolism which we propose for Cushing's disease and depression is manifested by the fact that responses to CRH in these disorders are in the opposite direction; e.g., an exaggerated ACTH response in Cushing's disease and a blunted one in depression. In all other diagnostic tests which have been utilized to differentiate depression from Cushing's disease, such as the dexamethasone suppression test and serial urinary free cortisol determinations, responses and/or levels in depression and Cushing's disease were in the same direction. Thus, the CRH stimulation test is in a unique position to assist in the differential diagnosis between depression and early Cushing's disease. Our data to date suggest that, as in most situations in clinical medicine, the CRH stimulation test will not be definitive in distinguishing depression from Cushing's disease. Thus, in studies of 30 depressed patients and 20 patients with Cushing's disease, three patients with Cushing's disease fall within the range of the ACTH responses which are noted in depression; similarly, two patients with depression fall within the range noted in patients with Cushing's

disease. Given this overlap between the two groups, conclusions about the diagnostic value of this test must await further extensive experience, although the data suggest that CRH testing should surpass the diagnostic accuracy of other tests now used to distinguish these two entities.

CRH in the Differential Diagnosis of Cushing's Disease and Other Causes of Cushing's Syndrome

Cushing's syndrome, as a spontaneous pathophysiological entity, can be divided into three types: Cushing's syndrome due to pituitary hypersecretion of ACTH (Cushing's disease), hypercortisolism secondary to ectopic secretion of ACTH, and the autonomous secretion of cortisol by an adrenal adenoma carcinoma. Thus, Cushing's syndrome can be divided into ACTH-dependent (the pituitary and ectopic ACTH secretion syndromes) and ACTH-independent (the cortisol producing adrenal neoplasm) subsets. The differential diagnosis between the two types of ACTH-dependent Cushing's syndrome is often difficult. In contrast, adrenal tumors are usually diagnosed radiologically or by ultrasound. The most sensitive procedure for this diagnosis is high resolution computerized axial tomography of the adrenal glands.

The CRH stimulation test appears to differentiate between Cushing's disease and the ectopic ACTH syndrome. Thus, in collaboration with Drs. G. Chrousos and his associates, we note that whereas almost all patients with Cushing's disease show exaggerated or robust ACTH response to CRH, patients with the ectopic ACTH syndrome generally fail to respond to CRH. Nine such patients were examined by us and four by other groups. Only one patient with ectopic ACTH secretion demonstrated responsiveness to CRH. This response was not found on repetition of the test. Since medical or surgical correction of the hypercortisolism in these patients is followed by a rapid return (within three days) of pituitary-adrenal axis responsiveness to CRH, hypercortisolism during testing is a prerequisite for assessing the response to CRH in these conditions.

Patients with ACTH-independent Cushing's syndrome had undetectable levels of plasma ACTH throughout the test and their plasma cortisol concentrations remained unaltered. Like patients with ectopic ACTH secretion, medical or surgical correction of the hypercortisolism was followed quickly by normalization of the CRH response.

We have concluded that CRH testing assists in the differential diagnosis between Cushing's disease, the ectopic ACTH syndrome, and adrenal causes of Cushing's syndrome. The available data, cited above, indicate about 1 of 44 (4.5%) false negatives and 1 of 13 (7.6%) false positives in differentiating pituitary from ectopic causes of Cushing's syndrome.

CRH Stimulation Test in Nelson's Syndrome

About 15% of patients who are treated for Cushing's disease show a marked increase in basal ACTH concentrations and hyperpigmentation associated with the pituitary tumor (Nelson's syndrome). We have shown that the microadenomas caused in Cushing's disease respond to CRH. Whether the tumors associated with Nelson's syndrome respond in a similar manner is unknown. In collaboration with Dr. Oldfield and his colleagues, we examined the plasma ACTH response to CRH in patients with Nelson's syndrome. All patients had tumors visible by a CT scan associated with elevated basal ACTH values, which showed marked rises after CRH.

Thus, the ACTH secreting adenomas in Nelson's syndrome, similar to the adenomas of Cushing's disease, respond to exogenous CRH.

It would represent an important advance in the treatment of Nelson's syndrome if the continuous infusion of CRH would "desensitize" the secretion of ACTH by these tumors. The phenomenon of pituitary desensitization was first described by Knobil et al, who observed that the pituitary secretion of luteinizing hormone and follicle stimulating hormone can be interrupted by the frequent or continuous infusion of LHRH. This has been found to have extensive clinical application in treatment of idiopathic precocious puberty, palliative therapy for prostatic carcinoma, and other conditions where suppression of the hypothalamic-pituitary-gonadal (HPG) axis is required, such as endometriosis. To examine the possibility of pituitary desensitization with CRH, we measured plasma ACTH concentrations in three patients who received continuous, maximal stimulatory infusions of oCRH for 24 hours. ACTH concentrations increased over the entire course of CRH administration and no evidence of desensitization was seen in any of these patients.

We can conclude from this that CRH stimulates ACTH in Nelson's syndrome. The tumors in this syndrome may be under the trophic influence of the hypothalamus, since the CRH neuron should be recovered from the suppressed state in these patients. ACTH response in Nelson's syndrome, compared to that observed in Cushing's disease, is probably related to the larger tumor size and the lack of hypercortisolism in these subjects. Continuous infusions of CRH for 24 hours failed to desensitize the pituitary secretion of ACTH in patients with Nelson's syndrome. The stimulation of ACTH released in these tumors by CRH implies the presence of CRH receptor. CRH antagonist, therefore, might prove useful in the management of those patients who frequently cannot be cured by current techniques.

The CRH Stimulation Test in the Differential Diagnosis of Adrenal Insufficiency

Adrenal insufficiency is divided pathophysiologically into two types: primary, when the adrenals are primarily responsible, and secondary, when either the pituitary gland of the hypothalamus fails. In collaboration with Dr. Schulte, CRH was administered to patients with adrenal insufficiency to determine whether the CRH stimulation test would be useful in the differential diagnosis of the condition. Twenty-three patients with primary and secondary adrenal insufficiency were studied. All but one were on replacement glucocorticoid therapy, which was discontinued from 12 to 60 hours before testing.

Patients with primary adrenal failure had high basal plasma ACTH levels and low basal cortisol values. Cortisol levels were low or undetectable throughout the test. Plasma ACTH values were markedly stimulated by CRH. Similarly, patients with secondary adrenal insufficiency also had low or undetectable basal levels of cortisol, and cortisol responses to CRH were generally minimal or absent. However, in contrast to the group with primary adrenal insufficiency, plasma ACTH concentrations were also low in these subjects, whereas the plasma ACTH responses were variable. Some patients had no ACTH responses to a CRH bolus, in contrast to the majority of patients who showed an early ACTH response similar to normal subjects. The response of these latter subjects, however, did not plateau but continued to increase during the test. The different ACTH response pattern in patients with secondary adrenal insufficiency cannot be accounted for by different clearance rates of CRH. All patients had immunoreactive CRH disappearance

curves from plasma which were similar to those of normal controls. We postulate that the patients who showed no ACTH response to CRH represent corticotroph cell failure (pituitary adrenal insufficiency); on the other hand, the patients who responded to CRH have endogenous CRH insufficiency (hypothalamic adrenal insufficiency). We conclude from these data that, in contrast to the experience with other hypothalamic releasing factors such as with LHRH and TRH, CRH may differentiate pituitary from hypothalamic causes of secondary adrenal insufficiency without a need for priming by multiple CRH injections.

Two patients with the rare syndrome of acquired idiopathic isolated ACTH deficiency had adrenal insufficiency with adrenal cortisol responses to a 48-hour ACTH stimulation test characteristic of the secondary form. These subjects also failed to respond to insulin-induced hypoglycemia and to vasopressin. These patients had undetectable plasma ACTH and cortisol responses to a bolus of CRF. Thus, they appeared to have pituitary rather than hypothalamic adrenal insufficiency.

Patients with corticotroph-sparing secondary adrenal insufficiency present a rare opportunity to define the regulatory mechanism of the HPA axis. It is these subjects in whom, after priming with ACTH, we replicated the physiological CRH secretion by administering eight pulses with hCRH spaced over the 24-hr period.

b. Arginine Vasopressin

We are continuing comprehensive investigation of arginine vasopressin function, addressing our studies to normal volunteers and to patients with psychiatric and medical illness alike. We have studied CSF vasopressin, the plasma vasopressin response to osmotic and non-osmotic stimulation, and the cognitive and behavioral responses to AVP analogue administration. As noted in previous reports, we have extensively studied AVP function in many psychiatric illnesses, including anorexia nervosa, primary affective disorder, and schizophrenia.

Our work in patients with anorexia nervosa revealed that these subjects respond to osmotic stimulation in a fashion not previously reported in individuals without documented hypothalamic tumors. Hence, the osmoreceptor seems totally functionally ablated in most underweight patients with anorexia nervosa, so that the neurosecretory neurons which produce and release AVP do so without heed to the osmotic milieu. This defect in the osmotic regulation of plasma AVP in anorexia nervosa is almost always associated with hypersecretion of AVP into the CSF. The hyperactivity of central AVP function in anorexia nervosa is of potential interest in light of the fact that AVP delays the extinction of learned aversive behaviors, and that a predominant symptom in anorexia nervosa is the heightened anticipation of the aversive consequences of eating. The possibility of increased central AVP in anorexia nervosa is also of potential interest in light of our demonstration that central CRH function seems augmented in these patients. AVP and CRH have been shown to be markedly synergistic at the level of the pituitary corticotroph cell and the same may be true in the CNS.

In one of the first publications positing a neuropeptide defect in major psychiatric illness, we postulated that AVP may be involved in some aspects of the symptom complex of affective illness. Hence, experimental data suggests AVP influences memory function, pain sensitivity, sleep, the synchronization of biologi-

cal rhythms, and the regulation of fluid and electrolyte balance, all of which are altered in affective disturbance.

In our studies in patients with depression, we noted that about 50% of bipolar patients show a partial diabetes insipidus (D.I.) with a diminished sensitivity of the AVP response to osmotic stimulation. In contrast, manic patients showed either augmented sensitivity of the AVP response to osmotic stimulation, or, in a few instances, an advance of the osmotic threshold for AVP secretion. This latter defect could result in water intoxication contributing to organic brain dysfunction. Measurement of CSF AVP in affective illness showed significantly reduced CSF AVP in depression and a somewhat augmented level of AVP in mania. Parenthetically, we noted a significant positive correlation between the sensitivity of osmotic regulation and the level of CSF AVP in our overall population of patients with affective disturbance.

To explore the possibility that lithium and carbamazepine, the two major agents used to treat and/or prevent bipolar illness, exert therapeutic effects by influencing vasopressin secretion or actions, we assessed the effects of these agents on the AVP response to osmotic stimulation. We noted that these agents each exert effects on AVP secretion in the direction of their demonstrated effects on AVP renal receptors. Hence, lithium, which is known to antagonize the renal response to AVP, significantly increased the sensitivity of the AVP response to osmotic stimulation. On the other hand, carbamazepine, which is known to be agonistic to the renal AVP receptor, significantly reduced AVP secretion to osmotic stimulation. Hence, by having one effect on secretion and an opposite effect on receptor function, these drugs could act to actually stabilize central AVP function in a manner analogous to the proposed actions of tricyclic antidepressants and other agents on regulated central neurotransmitter systems. From a clinical point of view, it was of interest that carbamazepine did not affect the osmotic threshold for AVP secretion. Hence, the case reports of water intoxication secondary to carbamazepine treatment would seem to represent an idiosyncratic response, since the osmotic threshold for AVP secretion was not lowered by treatment with this drug.

In light of the incidence of water intoxication in patients with chronic schizophrenia, we attempted to study osmoregulation and the secretion of CSF AVP in drug-free psychotic patients with schizophrenia. In contrast to the majority of patients with water intoxication, however, most of the patients we studied were relatively young with only a 3-10 year duration of illness. We noted that all 18 of our psychotic schizophrenic subjects showed normal parameters of AVP secretion; that is, osmotic sensitivity, threshold, and the precision of the osmostat. However, in contrast to these normal plasma responses, there was a significant reduction in the level of AVP secretion into the CSF in patients with schizophrenia. In light of the pronounced effect that AVP seems to exert on cognition in experimental animals, this putative central deficit of AVP in schizophrenia may bear some relationship to the thought disorder characteristic of this syndrome. In light of this hypothesis, it has recently been shown by the Wyatt group that the vasopressin analogue DDAVP seems efficacious in exerting a positive effect on some of the negative symptoms associated with the schizophrenic syndrome.

We recently reported on dose response studies conducted with the glucocorticoid antagonist RU-486 on AVP secretion. We note that RU-486 causes a significant increase in plasma AVP secretion, compatible with the idea that glucocorticoids exert chronic inhibition of central AVP function. In light of this finding, we shall explore whether the diminished AVP secretion into the plasma in patients with depression is related to the hypercortisolism classically associated with depression. We are currently investigating this possibility.

c. Somatostatin

We are continuing to collaborate with Dr. David Rubinow on studies of somatostatin secretion in the CSF. In collaboration with Dr. Rubinow, we have previously shown that somatostatin is significantly lower in a large group of drug-free unipolar and bipolar depressed patients compared to controls, and that psychoactive agents such as carbamazepine and zimelidine significantly influence the levels of somatostatin in CSF. Dr. Rubinow has also shown a significant relationship between CSF somatostatin secretion and the dexamethasone suppression status of patients, noting that somatostatin levels are lower in those who show the most pituitary-adrenal activation during the course of their depression. With regard to the question of a potential interaction between somatostatin and the HPA axis, we have shown that CSF somatostatin is markedly reduced in CSF of patients with active Cushing's disease. This suggests that hypercortisolism suppresses somatostatin secretion into the CSF, and it may account for the finding that patients with high basal cortisol values, or who fail to suppress with dexamethasone, show the lowest levels of somatostatin in the CSF.

d. Growth Hormone Releasing Hormone (GHRH)

Adjunct to our studies of the pathophysiology of vasopressin secretion and of HPA function in patients with anorexia nervosa, we have also attempted to explore the pathophysiology of the growth hormone hypersecretion, which is one of the classic neuroendocrine abnormalities associated with anorexia nervosa. To explore this question, we administered growth hormone releasing hormone to patients with anorexia nervosa studied longitudinally while chronically underweight and three weeks after correction of the weight loss. We noted that underweight patients showed elevated basal growth hormone levels which were associated with diminished plasma levels of somatomedin. The growth hormone response to GHRH in these underweight patients was exaggerated and correlated negatively with blood plasma somatomedin. We postulate that the elevated levels of GH and the hyperresponsive GH response to GHRH in patients with anorexia nervosa are secondary to the peripheral somatomedin deficiency which is characteristic of these patients. We postulate that the somatomedin deficiency may reflect either the hypercortisolism seen in these patients or the chronic inanition characteristic of their illness. The levels of basal GH and the GH response to GHRH completely normalized after the short-term correction of the weight loss, in association with full normalization of plasma somatomedin levels. Hence, the pathophysiology of the hypersecretion of GH in anorexia nervosa seems to reflect the deficient hepatic production of somatomedin, possibly secondary to either inanition or hypercortisolism in anorexia nervosa.

In previous reports we have detailed our findings regarding the effects of morphine and procaine on H-P-A axis function in depressed patients and controls. We have also administered insulin to induce hypoglycemia and have assessed the endocrine effects of this stimulus in both volunteers and patients with primary

affective disorder. As noted previously, insulin-induced hypoglycemic stress produces an ACTH response which significantly exceeds the ACTH response to maximal doses of CRH alone. This is compatible with other data that CRH is not the sole stimulus to the pituitary corticotroph cell but works in concert with other substances such as vasopressin and the catecholamines. In addition to ACTH, we also measured the plasma levels of vasopressin, oxytocin, and the catecholamines and are awaiting the analysis of this data. When a series of depressed patients was administered insulin in doses sufficient to produce significant hypoglycemia, it was noted that the ACTH response to this stimulus was significantly reduced in the depressed population. This pattern resembles that seen after the administration of CRH and most likely represents the effect of the hypercortisolism in these depressed patients to appropriately restrain the pituitary corticotroph cell in its response to hypoglycemia-induced CRH secretion.

We have also measured the plasma ACTH response to dexamethasone in control populations and in patients with depression. We attempted to correlate the ACTH response to dexamethasone with the measure of corticotropin releasing hormone in the CSF of our patients. The assessment of the ACTH response to dexamethasone in depressed patients is of considerable interest since there have been several conflicting reports in the literature concerning the pattern of responsivity in depressed patients. Moreover, there have been no previous studies in which the ACTH response to dexamethasone has been directly compared in depressed patients and controls. In collaboration with Dr. Alec Roy, we have shown that depressed patients show a clearly diminished ACTH response; i.e., suppression, compared to those seen in control subjects. It was reflected both in a significantly higher post-dexamethasone ACTH level in depressed patients as well as a smaller percentage fall in plasma ACTH after dexamethasone administration. Of theoretical interest is the observation that the post-dexamethasone change in plasma ACTH in depressed patients was inversely correlated with the level of CRH in the CSF. This finding is compatible with our previous work suggesting that hypercortisolism in depression reflects a defect of the hypothalamus resulting in the hypersecretion of endogenous CRH.

In previous reports we have detailed our studies concerning the secretion of basal and stimulated ACTH and CRH secretion into the CSF. In an additional study, in collaboration with Dr. Udelsman, we have developed a sensitive hemodynamic model in the anaesthetized cynomolgus monkey to characterize the cardiovascular effects of high doses of hCRH in the primate. Swan-Ganz thermodilution in arterial catheters were used to assess the hemodynamic effects of the central venous bolus injection of 90 μg/kg of hCRH. Immediately following direct administration, there was a marked fall in both peripheral vascular resistance and mean systemic blood pressure. Tachycardia was also noted, which was shown to be reflexic and subject to inhibition following antecedent beta-adrenergic receptor blockade. Cardiac output increased slightly, but the stroke volume and heart filling pressures remain unaltered. We demonstrated that this decrease in peripheral vascular resistance in the monkey is associated with the relatively selective dilitation of the superior mesenteric artery using electromagnetic flow probes. Similar observations have been made in the dog following administration of oCRH or related peptides that possess significant amino acid homology with CRH, such as sauvagine and urotensin I. hCRH or one of its analogues may thus have diagnostic and/or therapeutic uses as a peripheral hemodynamic regulator in man. It may prove useful as a selective splanchnic vasodilator during the radiologic or endoscopic

localization of occult G.I. tract bleeding. It may also be beneficial as a short-term afterload reducing agent in the management of patients in the setting of post-operative cardiac surgery or congestive heart failure, where an elevated peripheral vascular resistance compromises cardiac output.

Along with our interest in the physiology of stress, we have attempted to explore the physiologic relevance of glucocorticoid secretion to various aspects of the stress response. We have completed the first leg of this study. Thirty cynamolgus macacques were utilized; 15 were adrenalectomized and the other 15 received sham adrenalectomies. Each of the animals was then subjected to a surgical stress; namely, cholecystectomy. Several groups of animals were then sequestered; some received 1/10th glucocorticoid replacement dosage, while some received the normal replacement dosage and some received ten times the normal dosage. The results of the study show that those that were adrenalectomized and subjected to surgical stress showed no better cardiovascular adaptation with ten-fold glucocorticoid replacement dosage than with the normal replacement dosage. Hence, according to a variety of markers of cardiac function, including the cardiac output and cardiac index, the animals adapted perfectly well with maintenance glucocorticoid treatment. This supports the idea that glucocorticoids are permissive to normal cardiovascular adaptation during surgical and other stresses, and is in contrast to earlier concepts that pharmacologic glucocorticoid levels are required to adequately cope with stress. Thus, pharmacologic glucocorticoid replacement may not be indicated for patients with adrenal insufficiency undergoing surgical stress, thereby obviating risks such as poor wound healing and vulnerability to post-operative infection.

Significance to Biomedical Research and the Program of the Institute

The Section on Clinical Neuroendocrinology has attempted to extend current concepts regarding the physiology of neuroendocrine regulation in animal models and healthy volunteers, and to apply this understanding in an effort to unravel the pathophysiology of neuroendocrine disturbances in patients with major psychiatric and neuroendocrine illnesses.

Our work with corticotropin releasing hormone illustrates the implementation of our basic strategy for research. For instance, with respect to normal physiology, we advanced the first compelling data that CRH was of physiologic relevance to the regulation of the pituitary-adrenal axis in man. We also showed, however, that although CRH was of relevance to basal and circadian ACTH secretion, factors in addition to CRH seemed to be involved in stress-mediated ACTH secretion. Furthermore, our work strongly indicates that the pituitary corticotrophs all show a circadian rhythm in response to CRH. These studies, as well as our finding that the cortisol surge is associated with an increase in both the pulse frequency and magnitufe of neurosecretory episodes, has significantly added to the understanding of hypothalamic-pituitary-adrenal physiology in man.

Our studies of the pharmacokinetic and dose-response properties of ovine and human CRH led to clinical applications of this peptide, which have helped clarify some of the most elusive and difficult problems in neuroendocrinology. Thus, our work with CRH has helped to clarify the pathophysiology of hypercortisolism in depression and Cushing's disease, and has served as an aid in delineating the differential diagnosis. We have also shown that CRH is helpful in the differential

diagnosis of Cushing's disease from ectopic ACTH secretion and secondary from tertiary adrenal insufficiency. Moreover, studies with CRH in anorexia nervose and panic anxiety disorder have also helped to elucidate the pathophysiology of hypercortisolism in these disorders. Our data that the hypercortisolemic subgroups of psychiatric patients seem to show hypersecretion of endogenous CRF has implications for the fundamental pathophysiology of these disorders and supports the notion that they lie on a pathophysiological continuum. This idea is strengthened by findings such as that showing a positive correlation between CSF CRH and depression ratings in patients with anorexia nervosa. Parenthetically, our other studies in anorexia nervosa have significantly advanced our knowledge concerning the pathophysiology of several of the defects in neuroendocrine regulation seen in this entity, including those involving abnormalities in water metabolism and in growth hormone regulation.

Additional clinical studies of neurohypophyseal function have led to a further understanding of the variety of factors which influence osmotic and non-osmotic regulation of vasopressin secretion, and have led to systematic observations concerning alterations in the plasma and CSF secretion of this peptide in depression and schizophrenia, as well as in anorexia nervosa.

Several of our pre-clinical studies may also have clinical implications. For instance, we have demonstrated peripheral vascular effects of CRH which may have diagnostic implications for ascertaining the cause of occult G.I. bleeding and treatment implications as an overload reducing agent in the setting of post-operative cardiac surgery or congestive heart failure. Moreover, we have shown that pharmacological glucocorticoid replacement during surgical stress seems unnecessary to maintain cardiovascular integrity in the setting of adrenal insufficiency, with potential implications for the course of post-operative surgery, including the competence of wound healing and resistance to post-operative infection.

B. Proposed Course of Project

The Section on Clinical Neuroendocrinology addresses studies in several principal areas, whose future directions are outlined below.

1. Clinical and pre-clinical studies of normal physiology: Clinical studies concentrate on further characterizing ACTH secretion in normal volunteers. Projects underway include 24-hour sampling at 10-minute intervals to elucidate the pulse frequency of ACTH (and vasopressin) secretion; q30' hCRH administration for 24 hours to patients with hypothalamic-adrenal insufficiency to advance further data to suggest that the pituitary corticotroph shows a circadian rhythm and a sensitivity to CRH; studies with beta-adrenergic agonist given alone and in combination with CRH to assess the influence of catecholaminergic stimulation on corticotroph cell function. Pre-clinical studies are underway in primates to further characterize the regulation of CRH and vasopressin secretion into the CSF and the potential relationship between the CSF level of these peptides and pituitary-adrenal function. To accomplish this latter task, Dr. Kalogaras of our group has deployed a system to study primates which does not require chair restraint. Venous catheters are sutured into the external jugular vein and an Omaya reservoir is sutured into the dura of the cisterna magna; the catheters are tunneled underneath the skin to exit in the scapular plain and upon exiting are hooked up to a three-channel swivel system which directs the lines outside the cage. Each

animal is fitted with a comfortable jacket which does not permit access to the system. A variety of naturalistic and pharmacological studies are planned with this model to coordinate with the major thrusts of clinical research.

2. Studies on the physiology of stress: We plan to continue studies to explore factors which mediate stress-induced ACTH secretion. In addition to our current studies with metabolic stress, we are deploying an exercise paradigm in which control subjects exercise to 100% of their oxygen utilization and are then re-studied in a dose-dependent fashion; i.e., at 75% and 50% of maximal oxygen utilization. In addition to evaluation of neuroendocrine changes during exercise stress, we plan to study whether the dose-response curve has shifted for any of our clinical populations. We shall continue to explore immunoregulation in patients with depression, especially in relationship to glucocorticoid function. In this regard, we are working to develop an RIA for lipomodulin, the recent identified protein which plays an important role in mediating glucocorticoid effects on the inflammatory response. Pre-clinical studies will continue to study the physiological role of glucocorticoids during stress.

3. Clinical studies of primary affective disorder: A major focus in the coming year will be on studies of the 24-hour pattern of pulsatile ACTH secretion with q10' sampling. We predict that depressed patients will show an increased frequency of relatively low amplitude ACTH secretory episodes associated with relatively high frequency cortisol pulses. This will further substantiate our model of HPA dysfunction in depression. An additional new focus will be on exploring the acute and chronic effects of the anti-gulcocorticoid RU486. We predict that with antagonism of hypercortisolism in depressed patients, the ACTH responses to RU486 will be exaggerated, owing to hyperactivity of the hypothalamic CRH neuron. We also predict that euthymic patients with affective disorder will hyperrespond to RU486, since we postulate that such individuals will also show hyperresponsive pituitary-adrenal reaction to stress. In addition, we plan to study the response of euthymic patients to both insulin-induced hypoglycemia and to the exercise paradigm outlined earlier, to see if hyperresponsivity to stress is a trait marker for depressive illness.

Perhaps the most exciting new approach we will employ in the coming year is the assessment of the circadian pattern of neuropeptides in the CSF of depressed patients, in collaboration with Dr. Edward Oldfield. Studies with an indwelling lumbar catheter in place for 36 hours have already been conducted in normal controls without incident. We plan to measure peptides which normally show a circadian rhythm in CSF, whose levels may be abnormal in depression, and which seem functionally related to one another. Among those we propose to measure are CRH, ACTH, vasopressin, oxytocin, and somatostatin. Such an approach should provide considerably more useful information than single-point determinations obtained at 9 a.m. Parenthetically, our primate model should nicely complement this clinical project.

4. Clinical studies of anorexia nervosa: Neuroendocrine abnormalities constitute the cardinal biological manifestations of anorexia nervosa. Many postulate that this disorder is fundamentally a neuroendocrine disease. We have conducted three studies in anorexia nervosa which should substantially clarify the pathophysiology of several of the classic neuroendocrine abnormalities associated with this illness. Specifically, we have delineated the pathophysiology of osmo-

receptor dysfunction and CSF vasopressin secretion in these patients; we have strongly implicated CRH in the pathophysiology of their hypercortisolism; and we have identified the probable defect in the growth hormone hypersecretion widely described during the underweight phase of this illness. More recently, we have noted that CSF ACTH is markedly reduced in underweight anorectic patients, a finding which could reflect hyperstimulation of arcuate nucleus POMC cells by CRH.

It is important that we have the opportunity to follow up on these findings. For instance, we have not had the opportunity to explore whether there are defects in pulsatile plasma vasopressin secretion during the basal state or in non-osmotically stimulated vasopressin secretion. We would like to further characterize HPA function of these patients by measuring 24-hour rhythms for ACTH and vasopressin and administering challenges such as RU486. We would like to study the CSF circadian profile peptides in these patients. We are particularly intrigued by our findings that both central CRH and AVP function seem augmented in these patients. These peptides show marked synergy at the corticotroph cell and a combined defect could contribute to the pernicious quality of this illness. Such an hypothesis should be pursued in pre-clinical and clinical studies. Consequently, one of the proposed goals of the Section on Clinical Neuroendocrinology is to develop the means to apply our expertise to the study of patients with anorexia nervosa during various stages of their illness.

5. Cushing's disease and other neuroendocrine disorders: Our prior work with CRH has shown that the pathophysiology of the hypercortisolism in depression and Cushing's disease is distinct, and that the CRH test can be helpful in the differential diagnosis. We plan to continue to substantially enlarge our series of patients with depression and Cushing's disease to determine the prognostic accuracy of CRH stimulation in this differential diagnosis. We also plan to evaluate other means of aiding in the differential diagnosis of these two disorders, including the evaluation of responses to RU486, to vasopressin administration, and in free cortisol responses to CRH. Since almost all patients with Cushing's disease show a gradient with bilateral petrosal sinus catheterization, we also plan a study in patients with depression to evaluate the diagnostic accuracy of this procedure. Additional studies eventually planned in Cushing's disease patients include followup of CSF neuropeptide abnormalities with 36-hour circadian studies, and an evaluation of the functional characteristics of the osmoreceptor in these patients.

6. Future development of the laboratory: Our current radioimmunoassay laboratory has established and maintained radioimmunoassays for ACTH, vasopressin, oxytocin, hCRH, oCRH, and beta-endorphin. A radioreceptor assay for RU486 is now operational. We are currently working on developing assays for atrial natiuretic factor, neuropeptide Y, and lipomodulin. Current needs for this laboratory include the establishment of the capacity to perform bioassays to facilitate studies such as our search for "tissue" CRH factors, and the capacity to develop monoclonal antibodies, which are now showing promise via the monoclonal sandwich techniques of rapidly measuring neuropeptides with exceptional sensitivity. In addition, we strongly feel the need to develop an immunocytochemical laboratory, to deepen our capacity to ask questions and to interpret the already extensive body of data in neuropeptide abnormalities which we have reported in our clinical populations.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

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PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiology and Treatment of Menstrually-Related Mood Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH

P.P. Roy-Byrne, BPB, NIMH; P.W. Gold, BPB, NIMH; N. Rosenthal, CPB, NIMH; D. Goldstein, HEB, NHLBI; G. Merriam, ERRB, NICHD; R. Elin, CPD, CC, NIH; R. Reynolds, USDA; E. Bo, CC, NIH; A. Breier, NSB, NIMH; J. Calabrese, BPB, NIMH

COOPERATING UNITS (if any)

BPB, NIMH; Clinical Psychobiology Br., NIMH; Clinical Neurosci. Br., NIMH; Endocrinology & Reproduction Res. Br., NICHD; Hypertension-Endocrine Br., NHLBI; Clinical Pathology Dept, Clinical Center, NIH; U.S. Dept. of Agriculture

LAB/BRANCH

Biological Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The occurrence of dramatic changes in mood, behavior, cognition and somatic functioning in some women in relation to the menstrual cycle has recently been the focus of a great deal of public scrutiny. This project is designed to study the psychobiology and treatment response of women with well defined menstrually-related mood disorders. The longitudinal screening methods developed by this group are capable of distinguishing women with menstrually-related mood syndromes from those who only believe that they have such a syndrome. We are continuing to attempt to identify potential psychobiological correlates of menstrually-related mood changes by assaying serial blood samples for relevant hormones and by performing neuroendorcine, cognitive and electrophysiological tests during the symptom-free and symptomatic phases of the menstrual cycle. At present, no basal, diagnostic group or menstrual cycle phase-related abnormalities of anterior pituitary (gonadotropins, prolactin) ovarian (estradiol, progesterone) or adrenal (cortisol) hormones have been detected. An excess of women with menstrually-related mood disorders compared with controls did, however, show a TSH response to TRH outside the normal range. We are additionally performing double-blind controlled studies of several putative therapeutic agents, including progesterone and pyridoxine. Evidence to date suggests no superior benefit of progesterone or medroxy-progesterone compared with placebo.

The goals of this project are to detect and accurately describe menstrually-related mood disorders, explore their pathophysiology and response to pharmacological and environmental manipulation, and to document the relationship between reproductive endocrine change and disorders of mood as a way of further investigating the neurobiology of psychiatric illness.

PHS 6040 (Rev 1/84) GPO 904-917

I. Project Description

A. Objectives

This project has as its main intent the selection of subjects with carefully documented menstrually-related mood changes who can then undergo psychological and biological evaluation as well as participate in double-blind, placebo-controlled trials of several widely prescribed treatment modalities.

B. Methods Employed

1. Subjects

a. Subjects are self- and physician-referred women between the ages of 18 and 55 who complete visual analogue scale mood ratings twice daily for two months and, on the basis of these ratings, meet the following operational definition: a greater than 30% increase in self-rated anxiety or depression during the week prior to menses compared to the week following the cessation of menses in at least two consecutive cycles. All study participants are outpatients admitted to the Unit on Peptides Studies, Biological Psychiatry Branch.

b. Normal controls for this study include women with no complaints nor evidence of menstrually-related mood disorder and who are without primary psychiatric illness, and women who have complaints of, but no visual analogue scale evidence of, menstrually-related mood changes.

2. Procedures

Phase 1. An extensive screening phase that has been described in detail in Project Z01 MH 00180-02 BP.

Phase 2. This is an intensive psychobiological evaluation phase for patients meeting entry criteria for the study.

a. Patients are given a thorough physical and laboratory examination in order to rule out the presence of unknown medical illness.

b. Plasma steroid and peptide studies involving serial blood samples are being conducted, as previously described with Drs. George Merriam (ERRB, NICHD) and David Goldstein (HEB, NHLBI). Methods and rationales for ongoing studies of plasma hormones, catecholamines and magnesium (Dr. Ronald Elin, CPD, CC), as well as TRH (Dr. P.P. Roy-Byrne, BPB, NIMH) and CRH (Dr. P.W. Gold, BPB, NIMH) stimulation tests in women with menstrually-related mood disorders and controls have been described in Project Z01 MH 00180-02 BP. Finally, measurements of plasma pyridoxal phosphate are being conducted by Dr. Robert Reynolds (USDA). We are, in addition, performing the following studies:

1) Psychometrics: In addition to a cognitive and mood self-rating battery already described, we are evaluating the frequency of and response to life events over the course of the menstrual cycle (M.C. Hoban, BPB, NIMH).

2) Diet: In collaboration with Dr. Norman Rosenthal (CPB, NIMH) and Ernestine Bo (CC, NIH), we are evaluating dietary patterns over the course of the menstrual cycle in order to test hypotheses about altered carbohydrate or salt intake in menstrually-related mood disorders.

3) Glucose tolerance: Because of reports of altered carbohydrate tolerance in premenstrual syndrome, we are performing glucose tolerance tests in patients with menstrually-related mood disorders in both cycle phases.

4) Stress testing: As patients report a menstrually-related alteration in perception of events or adaptation to events, we are beginning to assess cognitive and electrophysiologic response to stress, in collaboration with Dr. Alan Brier (NSB, NIMH), employing a white noise, negative performance feedback paradigm.

5) Immune system: In light of reports of both altered T-cell function as a function of estrogen levels and abnormal allergic responses in women with premenstrual syndrome, we are investigating T-cell function in relation to menstrual cycle phase in women with premenstrual syndrome and normal controls, in collaboration with Dr. Joseph Calabrese (BPB, NIMH).

Phase 3. This is a multi-modality treatment phase for patients who have completed Phase 2.

1) Pharmacologic: Double-blind, placebo-controlled crossover evaluations of progesterone, medroxyprogesterone acetate, pyridoxine and carbamazepine are currently being conducted. The first three agents mentioned are cited as effective in the literature but have not been systematically demonstrated to be more effective than placebo in studies to date, largely as a function of methodological flaws which render the results of these studies ungeneralizable. Carbamazepine has been successfully used in the treatment of premenstrual psychomotor seizure-related behavioral syndromes as well as major affective disorder.

C. Findings

Forty-four percent (94/215) of subjects who have completed three months of daily ratings met operational criteria for a menstrually-related mood disorder. Thirty-five women and twelve controls have completed the second phase of the protocol, participating in one month of frequent blood drawings for assessment of endocrine variation over the course of the menstrual cycle, as well as in assessment of cognitive function and response to dexamethasone and TRH infusion during the symptomatic and symptom-free phases of the cycle. Preliminary evaluation of endocrine factors in women with premenstrual syndrome reveals no inadequate corpus luteal activity and no systematic hormonal deficiency. Confirmation of these findings must await hormonal assessment of the remaining subjects and controls. Assessment of TSH response to TRH during the follicular and luteal phase of the cycle showed that eight out of twelve patients with menstrually-related mood disorder versus one out of ten controls manifested a peak delta-TSH response that was outside of the established normal range. The significance and consistency of these findings remain to be established. Assessment of white cell, red cell and plasma magnesium levels over the course of the menstrual cycle revealed significant reductions in the plasma calcium:magnesium ratio in thirteen patients with menstrually-related mood disorder during the luteal phase compared with six controls. No systematic abnormality of mononuclear cell magnesium or calcium was demonstrated. Assessment of pyridoxal phosphate levels by Dr. Robert Reynolds (USDA) has demonstrated no significant differences between patients and controls. Assessment of plasma catecholamines showed no systematic or symptom-related variation. Perceptions of cognitive performance varied considerably with menstrual cycle phase, al-

though actual performance revealed no systematic menstrual-cycle phase-related alterations. Perceptions of life events appear to undergo dramatic menstrual cycle-related changes, with patients more likely to assess offense as negative during the luteal phase.

Twelve people have entered the treatment phase of the study and five have completed the ten-month trial at this point. No superiority of either progesterone or medoxyprogesterone has been demonstrated.

D. Proposed Course of Project

To date, over 500 women have requested to be participants in our project and are at various stages of evaluation. With a group of well-defined patients, we hope to explore the natural course of menstrually-related mood disorders as well as their phenomenology and biological correlates in relation to treatment response. Early endocrine findings will be pursued and specific hypotheses regarding the etiology of premenstrual syndrome (e.g., endorphin addition/withdrawal, carbohydrate intolerance, electrolyte dysregulation) will be tested with endocrine challenge studies. Treatment protocols will be completed, and evaluation of putative therapeutic agents will be undertaken. Cognitive testing will be continued with the addition of distractors during testing. In addition, we wish to expand our investigation of the effects of menstrual phase on mood to include patients hospitalized at the Clinical Center with major affective disorder, panic anxiety disorder, anorexia-bulimia, as well as patients with hereditary angioedema. Our early experience with a number of women with these disorders suggest that symptoms may be exacerbated or may cluster during the premenstrual period; these clinical impressions require prospective confirmation.

E. Significance to Biomedical Research and the Program of the Institute

Despite the current lack of clear understanding of the nature of the relationship between mood disorders and the menstrual cycle, numerous studies of this phenomenon suggest its importance to the psychiatrist on many levels: practically (as a problem about which the psychiatrist may be called to consult or as a factor which may influence the course of the treatment of patients); heuristically (as a model for learning about state changes, a process of clear relevance to studies of other mood state disorders such as manic-depressive illness or panic anxiety disorder); and conceptually (as a potential means for providing biological-phenomenological isomorphs and further understanding the role of entrainment in episodic or cyclic psychiatric disorders). Menstrually-related mood disorders in their own right are important to better understand, if only for the fact that there are large numbers of women who feel that they suffer from such syndromes and seek treatments that are unproved and potentially dangerous. In addition, it would appear that menstrual cycle phase is a variable that has been all too frequently ignored in studies of traditional psychiatric and medical illnesses. It is our belief, therefore, that this project will provide information that will be of immediate clinical relevance and that will further our understanding of the complex relationship between endocrine system activity and mood.

Publications

Rubinow, D.R.: PMS: Practical and ethical aspects of pharmacotherapeutic evaluation. In Ginsberg, B. and Frank-Carter, B. (Eds.): Legal and Ethical Implications of the Biobehavioral Sciences. New York, Plenum Press, in press.

Rubinow, D.R., Roy-Byrne, P.P., and Hoban, M.C.: Menstrually-related mood disorders. In Dawood, M.Y., McGuire, J.L., and Derners, L.M. (Eds.): Premenstrual Tension and Dysmenorrhea. Baltimore, Urban and Schwarzenberg, 1985, pp. 27-40.

Rubinow, D.R., Roy-Byrne, P.P., Hoban, M.C., Grover, G. and Post, R.M.: Premenstrual syndromes: past and future research strategies. Can. J. Psychiatry, in press.

Rubinow, D.R., Roy-Byrne, P.P., Hoban, M.C., Grover, G., and Post, R.M.: Menstrually related mood disorders. In Osofsky, H.O. and Blumenthal, S.J. (Eds.): PMS: Current Findings and Future Directions. Washington, D.C., American Psychiatric Press, 1985, pp. 1125-1136.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00181-02 BP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormonal Studies of Affective Disorder

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH

D. Pickar, M.D., NSB, NIMH; A. Doran, M.D., NSB, NIMH; G. Merriam, M.D., ERRB, NICHD; T. Insel, M.D., LCS, NIMH; W. Kaye, M.D., LPP, NIMH; M. Kling, M.D., BPB, NIMH; P. Sunderland, M.D., LCS, NIMH; R. Joffe, M.D., BPB, NIMH

COOPERATING UNITS *(if any)*

Clinical Neuroscience Branch, NIMH; Laboratory of Clinical Science, NIMH; Endocrinology & Reproduction Research Branch, NICHD; Laboratory of Psychology and Psychopathology, NIMH;

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Biological Psychiatry Branch

SECTION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Endocrine studies were performed to assess alterations in somatostatin and cortisol activity in relation to affective illness. The relationship between depression-related cognitive disorder and endocrine dysregulation has also been examined.

A) Somatostatin - With recently learned assay techniques and in collaboration with Drs. Pickar and Doran, we confirmed a predicted relationship between post-dexamethasone cortisol and CSF somatostatin. This relationship may provide a biochemical explanation for psychiatric disorder-related cortisol abnormalities. We have identified neuroleptic-induced reductions in CSF somatostatin in 16 schizophrenic patients to complement our previous finding of carbamazepine-induced elevations of CSF somatostatin. Studies in underweight and re-fed anorectic patients revealed no abnormalities of CSF somatostatin, while reductions were observed in patients with Alzheimer's syndrome. Work is currently underway to clarify the significance of these findings, as well as to further delineate the factors regulating hypothalamic somatostatin secretion.

B. Cortisol - We have investigated a number of measures of hypothalamic-pituitary axis activity including response to dexamethasone, mean urinary free cortisol excretion and the relationship between plasma cortisol levels and affective state in relation to a variety of biological and phenomenological features of depression. We have demonstrated clearcut elevations in CSF cortisol in depressed patients relative to normal controls and patients during the improved state. Additionally, we have observed elevations of mean urinary free cortisol excretion during treatment with the anticonvulsant psychotropic agent, carbamazepine. The goals of the above-listed projects will be to expand our understanding of known relationships between depression and endocrine function.

PHS 6040 (Rev. 1/84) GPO 904-917

I. Project Description

A. Objectives

The goal of this project is to study measures of two endocrine systems, somatostatin and cortisol, in patients with affective illness in order to expand our understanding of the mechanisms and significance of reported abnormalities in these systems in affective illness.

B. Methods Employed

1. Subjects

a. Subjects are inpatients on NIMH clinical units meeting criteria for major depressive disorder, schizophrenia, Alzheimer's disease or anorexia nervosa. Patients with Cushing's disease who are inpatients on a NICHD unit have also been studied.

b. Normal controls for CSF and hormone infusion studies are subjects participating in the normal volunteer program at the NIH.

2. Procedures

Lumbar punctures are performed to obtain CSF samples for somatostatin, cortisol and other related CNS peptides/neurotransmitters. Additionally, blood and urine samples are obtained for measurement of cortisol. Dexamethasone suppression tests are performed in patients while medication-free and while on treatment with carbamazepine. Infusions of oxytocin and vasopressin have been performed in order to assess the effects of these hormones on cortisol secretion and cognitive functioning.

C. Findings

As previously reported in project Z01 MH 00181-01 BP, evidence of affective state-related cortisol dysregulation has been found with increased mean urinary free cortisol excretion during depression, increased CSF cortisol during depression, and significant relationships between plasma cortisol and affective state observed. Cognitive studies previously described have also continued to reveal an affective state-related cognitive disorder. We have expanded our investigations of CSF somatostatin in affective disorders by studying groups of patients with Alzheimer's disease (Dr. Pearson Sunderland), anorexia nervosa (Dr. Walter Kaye) Cushing's disease (Dr. Mitchell Kling) and schizophrenics (Dr. Alan Doran). While no alteration of CSF somatostatin was observed in patients with anorexia nervosa during the starved or re-fed state, significant reductions were observed in patients with Alzheimer's disease consistent with other reports appearing in the literature. Additionally, preliminary evidence suggests that CSF somatostatin is reduced in patients with Cushing's disease. With Drs. Pickar and Doran, we demonstrated that CSF somatostatin was significantly reduced in schizophrenic patients during treatment with fluphenazine compared with medication-free patients. These clinical findings provide support for the reported ability of dopamine blockers to reduce somatostatin secretion *in vitro*. Finally, in collaboration with Dr. Russell Joffe, we have demonstrated a significant relationship to exist between CSF somatostatin and plasma T_4 and free T_4 levels that persists even following pharmacologic perturbation with carbamazepine. This observation provides indirect support for the observed mutual regulation of somatostatin and thyroid hormone.

Consistent with our earlier findings, we have continued to observe increased mean urinary free cortisol excretion and reduced levels of CSF somatostatin fol-

lowing treatment with carbamazepine. These findings are consistent with a central stimulating or dysinhibiting effect on hypothalamic-pituitary-adrenal axis activity and may suggest a role for somatostatin in carbamazepine-induced escape from dexamethasone suppression.

D. Proposed Course of Project

We hope to:

1. Expand the known clinical concomitants of various measures of hypothalamic-pituitary-adrenal axis dysregulation;
2. Establish, in collaboration with Dr. Thomas Insel, the impact of reduced peripheral and CSF levels of somatostatin on pituitary ACTH release;
3. Explore, with Dr. George Merriam, those factors regulating hypothalamic somatostatin activity;
4. Examine the effects of electrical kindling and learned helplessness on brain somatostatin in rodents;
5. Compare the concentration of somatostatin in post-mortem brain regions of schizophrenic patients, suicides and controls;
6. Pursue in a larger population of depressed patients the memory enhancing effects of vasopressin infusion;
7. Investigate the neuroendocrine concomitants of impaired cognitive performance observed on several measures of cognition (Halstead Category Test, Face Test).

E. Significance to Biomedical Research and the Program of the Institute

Depression-related dysregulation of somatostatin and cortisol may provide a window into the central neurochemical lesions responsible for depression. Further, specific behavioral of physiological disturbances (e.g., cognitive impairment or cortisol dysregulation) may be products of abnormal neuroendocrine activity. It may prove to be the case that depression-related reductions in somatostatin are mechanistically relevant to depression-related disturbances in hypothalamic-pituitary-adrenal activity, the most commonly reported biological abnormality in depression. Further study may not only enhance our knowledge of the neurobiology of depression, but may, as well, generally inform us about the relationship between hormones and human behavior.

Publications

Rubinow, D.R., Doran, A.: CSF somatostatin in psychiatric disorders. Biol. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00182-02 BP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behavioral Medicine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH

Dr. Russell Joffe, Biological Psychiatry Branch, NIMH;
Dr. David Pickar, Clinical Neuroscience Branch, NIMH;
Dr. William Sindelar, Surgery Branch, NCI;
Dr. Philip Schneider, Surgery Branch, NCI;
Dr. Allan Mirsky, Lab. of Psychology & Psychopathology, NIMH;
Dr. Clifford Lane, Lab. of Immunoregulation, NIAID;

COOPERATING UNITS *(if any)*

BPB, LPP, CPB, NSB, NIMH; SB, MB, PB, D, NCI; LIR, LCI, NIAID; CCM, PHARM, CC; ARB, CEB, DDB, OD, NIADDKD; CB, MDB, NHLBI

LAB/BRANCH

Biological Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Advances in neuroscience as well as the appreciation of the often unrecognized role of behavior and disturbances of behavior in many medical disorders has led to the creation of a behavioral medicine research program based in the Consultation-Liaison Service of the Intramural Program. Ten protocols are currently active or planned investigating the mood, cognitive and behavioral concomitants of cancer of the pancreas, acquired immune deficiency syndrome, chest pain with normal coronary arteries, interferon therapy, metoclopramide administration, steroid therapy and thyroid hormone replacement and withdrawal. These protocols will address such areas as: a) the effects of previous psychiatric history on the psychiatric morbidity associated with certain diseases and their treatment; b) the psychiatric phenomenology of certain diseases and their treatment; c) the treatment response characteristics of psychiatric disorders associated with diseases or their treatment; d) biochemical factors that may serve as predictive diagnostic markers for illness or for treatment-associated mood/behavioral or cognitive syndromes. The goals of this project will be to address these areas of investigation in selected patient populations at the NIH Clinical Center.

Significant findings to date include demonstration of cognitive deficits in patients with acquired immune deficiency syndrome who have no other evidence of neurologic involvement, establishment of endogenous depression as a frequent presenting symptom in cancer of the pancreas as opposed to gastric cancer, observation of significant fluctuations in mood attendant to administration of alternate day steroids in patients with lupus and identification of significant reductions of depression and anxiety following successful treatment of cystic acne.

Other Professional Personnel (continued)

Dr. Anthony Fauci, Lab. of Clinical Investigation, NIAID
Dr. Dan Longo, Medicine Branch, NCI
Dr. Henry Masur, Critical Care Medicine, CC
Dr. James Hathorn, Pediatric Oncology Branch, NCI
Dr. Garry Peck, Dermatology Branch, NCI
Dr. Patricia Petrick, Clinical Endocrinology Branch, NIADDKD
Dr. Jacob Robbins, Clinical Endocrinology Branch, NIADDKD
Dr. Robert Golden, Clinical Psychobiology Branch, NIMH
Dr. Jeffrey Hoeg, Molecular Disease Branch, NHLBI
Dr. Bryan Brewer, Molecular Disease Branch, NHLBI
Dr. Daniel Hommer, Clinical Neuroscience Branch, NIMH
Dr. Steven Paul, Clinical Neuroscience Branch, NIMH
Dr. Owen Wolkowitz, Clinical Neuroscience Branch, NIMH
Dr. Jay Hoofnagel, Digestive Diseases Branch, NIADDKD
Dr. E. Anthony Jones, Digestive Diseases Branch, NIADDKD
Dr. Pierre Renault, Associate Director, NIADDKD
Dr. George Tsokos, Arthritis & Rheumatism Branch, NIADDKD
Dr. Stanley Pillemar, Arthritis & Rheumatism Branch, NIADDKD
Dr. Peter Roy-Byrne, Biological Psychiatry Branch, NIMH
Dr. Richard Cannon, Cardiology Branch, NHLBI

I. Project Description

A. Objectives

This project has as its main intent the identification of mood and cognitive factors that appear in the context of specific medical illnesses and their treatment, determination of the relationship between these factors and both the primary medical disorder and prior psychiatric history and utilization of the occurrence of these factors in a medical context as models for the production of similar symptoms in a primarily psychiatric context.

Protocols

Active:

1) Clinical and biological features of mood and cognitive disorders in patients with carcinoma of the pancreas.
2) Neuropsychiatric dysfunction in patients with acquired immune deficiency syndrome (AIDS)
3) Psychiatric effects of treatment of cystic acne with 13 - Cis-retinoic acid
4) Longitudinal assessment of cognitive and mood disorders in patients with type V hyperlipoproteinemia
5) Mood and cognitive effects of interferon administration in patients with chronic active hepatitis
6) A prospective study of the behavioral, cognitive and neurochemical effects of chronic systemically administered corticosteroids
7) The effect of thyroid replacement and withdrawal on cognition and mood in patients with carcinoma of the thyroid

Written:

1) Assessment of neuropsychiatric concomitants of metoclopramide administration
2) Chest pain with normal coronary arteries: evaluation of response to sodium lactate in relationship to primary anxiety disorders
3) Evaluation of the somnographic, affective and rheumatological characteristics of patients with fibrositis

B. Methods Employed

1. Subjects

a. Subjects are NIH patients who are referred for participation in these protocols by collaborators from the Institute responsible for the primary care and treatment of these patients.

b. Controls for the individual studies are selected in a way that allows for stratification of populations with respect to the relevant variables under study. For example, assessment of the incidence of endogenomorphic depression in patients with carcinoma of the pancreas requires utilization of a patient control population with other intra-abdominal malignancies.

2. Procedures

a. Psychiatric Diagnostic Evaluation. The primary methodology employed is that of evaluating the psychiatric history of all subjects and their families utilizing a semistructured psychiatric interview, the Schedule for Af-

fective Disorders and Schizophrenia (SADS-L), which provides information from which an RDC diagnosis can be made.

b. Longitudinal Evaluation. Most studies utilize a "self as own control" design employing longitudinal assessment of mood ratings, physical symptoms, and cognitive performance. Measures of mood include the Beck Depression Inventory, the State-Trait Anxiety Inventory, the Symptom Check List-90, and a number of hundred millimeter line visual analogue scales of mood. Visual analogue scales are also used to assess the presence and severity of physical symptoms. Cognitive measures include the Mini-Mental Status Exam, the Halstead-Wepman Test, and an extensive battery of neuropsychological tests developed by Kathleen Squillace (LPP, NIMH). In most instances, episodic observer ratings are augmented by daily subjective ratings. By these means, the time course of the development of cognitive or affective changes can be more precisely defined.

c. Laboratory Assessment. Urine and/or blood samples are collected in order to permit evaluation of those biological substances believed to be related to the development of affective or cognitive disturbances.

C. Findings

Preliminary findings include:

1. Marked diminution in anxiety and depression in patients successfully treated for cystic acne;
2. Depression as a frequently associated and often presenting symptom complex in patients with carcinoma of the pancreas compared with patients with gastric carcinoma;
3. The existence of moderate impairment of generalized intellectual function and evidence of specific cognitive dysfunction in patients with AIDS compared with controls;
4. The occurrence of depression as the most disabling adverse effect of interferon treatment for chronic active hepatitis;
5. The induction of prominent mood swings in patients with quiescent lupus as a function of treatment with alternate-day steroids;
6. Precipitation of a marked depressive syndrome in normal volunteers taking a short-term course of prednisone.

D. Proposed Course of Project

The active and proposed studies noted above will be continued until adequate numbers of subjects are obtained. Early findings in these studies should permit design of focused investigations of the neurobiology of specific mood, behavioral and cognitive disorders.

E. Significance to Biomedical Research and the Program of the Institute

The studies in this project are hypothesis-generating as well as hypothesis-testing. Thus, they should not only help to expand the behavioral phenomenology of many medical disorders, but should, as well, suggest optimal studies for the application of modern neuroscientific techniques to disorders of regulation of mood and cognition. The utilization of medical disorders as models for the development of mood and cognitive disturbances in the context of biological

dysregulation should clarify the meaning of these biological alterations already observed in psychiatric disorders.

Publications

Pincus, H. and Rubinow, D.R.: Research at the interface of medicine and psychiatry. In Pincus, H. (Ed.): The Integration of Neuroscience and Psychiatry. Washington, D.C., American Psychiatric Press, Inc., in press.

Rubinow, D.R.: The psycho-social impact of AIDS. Topics in Clinical Nursing. July, 1984, pp. 26-30.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00124-08 BP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Action of Lithium in the Treatment of Affective Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH

COOPERATING UNITS *(if any)*

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Biological Psychiatry Branch

SECTION

Unit on Behavioral Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been incorporated into Project #Z01 MH 00147-10 BP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

.Z01 MH 00147-10 BP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Effects of Brain Peptides and Other Psychoactive Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH

P.B.S. Clarke, Visiting Fellow, BPB, NIMH; N. Ostrowski, PRAT Fellow, BPB, NIMH; H. Wheeling, PRAT Fellow, BPB, NIMH; C.C. Chiueh, Staff Fellow, NINCDS; S.R.B. Weiss, Staff Fellow, BPB, NIMH; C.B. Pert, Chief, Sect. on Brain Biochemistry, NSB, NIMH; J. Glowa, Staff Fellow, NSB, NIMH; D. Friedman, Staff Fellow, LN, NIMH; K. Rice, LC, NIADDK; A. Fabbri, Univ. of Rome, Italy; T. Seeger, Pfizer Central Research, Groton, Ct.

COOPERATING UNITS (if any)

LN, NSB, NIMH; LC, NIADDK; NINCDS; Univ. of Rome, Italy; Pfizer Central Research, Groton, Ct.

LAB/BRANCH

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NIMH, NIH, Bethesda, Maryland 20205

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5	4	1

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nicotine receptors were visualized in rhesus monkey brain with autoradiographic procedures. Binding was heaviest in various thalamic nuclei. Relatively heavy labeling was also found in the geniculate nuclei and substantia nigra. In cortex, nicotine receptors were restricted to layer II. In rat nicotine receptors were found on both the terminals and cell bodies of dopamine neurons. In the interpeduncular nucleus nicotine receptors were localized both presynaptically and postsynaptically to Ach neurons. Lesion studies revealed that dopamine perikarya do not contain opiate receptors. Opiate receptors in the substantia nigra appear to be located on terminals of striato-nigral afferents. ^{3}H-cyclo-foxy (an opiate ligand that can also be fluorinated) was shown to produce identical binding patterns in rat brain following in vivo or in vitro application. This novel ligand has potential use in PET applications. In hamsters, the sexually dimorphic nucleus in the male showed denser opiate receptor binding than in female. Pattern differences also appeared in opiate receptor binding in the hypothalamus between sexes. In vivo autoradiographic procedures revealed differences in ^{3}H-diprenorphine binding densities between control males and mated males in various hypothalamic nuclei, suggesting enhanced release of endogenous opiates during mating. Electrical stimulation of the arcuate nucleus also produced decreased binding of ^{3}H-diprenorphine in terminal areas of the beta-endorphin system suggesting release of beta-endorphin. Such stimulation was also accompanied by a naloxone reversible analgesia. Morphine raised aversive brain-stimulation thresholds in the mesencephalic reticular formation while neurotensin lowered thresholds. Autoradiographic analyses revealed exceptionally heavy binding of salmon calcitonin in the nucleus accumbens. Calcitonin receptors were found to be postsynaptic to dopamine (DA) terminals in the n. accumbens but located on DA cell bodies in the substantia nigra. Injections of salmon calcitonin into the n. accumbens produced profound depression of locomotor behavior. Phencyclidine was found to enhance locomotor output through the n. accumbens by a DA-independent mechanism.

I. Project Description

A. Objectives

1. Nicotinic Receptors in the Brain

Biochemical, electrophysiological, and behavioral evidence suggests that nicotine acts in the brain. These effects are probably mediated through specific nicotine receptors. Alpha-Bungarotoxin (BTX), which blocks nicotinic cholinergic transmission at the neuromuscular junction, has been widely used to label central putative nicotinic cholinergic receptors. Recently, we have demonstrated stereospecific, saturable and reversible binding of tritiated nicotine to rat brain sections, which is of high affinity and is selectively displaced by nicotinic agonists including acetylcholine. We have also shown that the anatomical distribution of nicotinic binding sites labeled either with tritiated nicotine or with tritiated acetylcholine differs markedly from the pattern of BTX labeling in adjacent sections. Agonists appear to bind to central receptors resembling those at autonomic ganglia, and BTX binding sites also seem to represent pharmacological sites of action in certain brain regions.

2. Presynaptic Localization of Nicotinic Binding Sites in the Interpeduncular Nucleus (IPN)

The IPN lacks cholinergic perikarya but receives a massive cholinergic input, carried almost exclusively in the fasciculus retroflexus of Meynert (FR). Both tritiated nicotine and ^{125}I-BTX bind densely within IPN, whereas muscarinic receptor binding is low. Electrophysiological data indicate that the FR cholinergic terminals may possess presynaptic nicotinic cholinoceptors regulating the release of acetylcholine. Such a phenomenon has yet to be described in the brain. The lack of axo-axonic contacts among FR afferents, together with the absence of early transsynaptic degeneration in IPN following FR lesions, make this an appropriate model system for detecting presynaptic nicotine binding sites; specifically, any loss of receptors in IPN following shortly after bilateral FR lesions can be attributed to the disappearance of receptors located on the presynaptic membrane of FR afferents.

3. Autoradiographic Distribution of Nicotinic and Muscarinic Cholinoceptors in Monkey Brain

There are no published descriptions of cholinoceptive binding in monkey brain; recently, the neuroanatomical distribution of cholinergic neurons has been described using ChAT antibodies. In view of the cholinergic neuropathology associated with Alzheimer's disease (presenile dementia), we sought to characterize nicotinic and muscarinic binding sites in monkey brain and to determine their anatomical distributions. Brain sections from rhesus monkeys were processed for *in vitro* receptor autoradiography, using tritiated nicotine and QNB (a muscarinic ligand).

4. Nicotinic Cholinoceptors in the Nigrostriatal Dopamine System

Dopaminergic (DA) cells of the substantia nigra pars compacta (SNC) contain AchE but not ChAT and hence may be cholinoceptive. The presence of muscarinic cholinoceptors on these DA cells is controversial, but tritiated nicotine binds densely within the SNC. We have previously shown that systemic nicotine increases DA neuronal firing rate via a central action. In order to determine whether nicotine receptors in the SNC are located specifically on DA cells, rats were injected unilaterally with 6-hydroxydopamine given into the ascending nigrostriatal tract.

Five weeks later, striatal DA content was assayed, and brain sections were taken for ^{3}H-nicotine receptor autoradiography.

5. Nicotinic-cholinergic Transmission in SNC

Our autoradiographic and lesion experiment confirmed the presence of nicotine receptors on DA SNC neurons. Two tegmental nuclei (pedunculopontine - PPN; dorsolateral tegmental - DLTN) project to SNC and each contains a cholinergic cell body group. Electrical excitation of the PPN region elicits a monosynaptic excitation of SNC DA cells; the identity of the transmitters has not been determined. In order to determine whether this excitation derived from excitation of PPN neurons rather than axons of passage, we injected kainic acid into or near the PPN while recording extracellularly from DA SNC single units.

6. Site of Action of Opiate Modulation of Dopamine

Endogenous opioids and opiate receptors are found in close proximity to dopamine neurons and terminals in the brain. Pharmacological data suggest that opiate receptors near or on dopamine neurons in the substantia nigra pars compacta and pars reticulata may be one site of opiate effects on dopamine-linked activity and positive reinforcement. We conducted autoradiographic studies to determine whether opiate receptors were localized on dopamine cell bodies or on afferents to the substantia nigra in rats.

Selective lesions of dopamine neurons and nigral afferents were performed. The dopamine neurotoxin, 6-hydroxydopamine, was injected into the medial forebrain bundle to eliminate dopamine neurons in the substantia nigra. Another group of animals received kainic-acid lesions of the striatum, which sends projections to the nigra. Two more groups received electrolytic lesions of either the internal capsule or the globus pallidus, regions that also send fibers to the substantia nigra. All lesions were unilaterally placed so that each animal served as its own control. Slide-counted brain sections from the lesioned rats were incubated *in vitro* with ^{3}H-opioids and processed for film autoradiography.

7. Strategy for Elucidating the Contribution of Opiate Receptor Subtypes in Morphine's Behavioral Effects

Assessing the contribution of subtypes of opiate receptors in mediating effects of narcotics (i.e., analgesia, locomotor activation, physical dependence, reinforcement, tolerance) has proven difficult, since there are few selective opiate receptor agonists and no opiate receptor antagonists specific for mu, delta, kappa, or epsilon receptors. Dr. Kenner Rice has synthesized alkylating agents which, according to autoradiographic studies, selectively alkylate mu opiate receptors (BIT) of delta opiate receptors (FIT). The usefulness of these compounds is limited since: a) they cannot be administered systemically into live animals; b) they produce profound aggression after i.c.v. injections in rats; c) they produce long-lasting toxic effects; and d) have agonist-like properties after acute administration. In collaboration with Dr. J. Glowa, we tested BIT to determine whether a procedure could be developed that would be amenable to mapping opiate receptor subtypes in brain and their role in narcotic-produced effects on behavior.

Two groups of rats received daily local infusions of morphine into the ventral tegmental area, which produced the well-described locomotor stimulatory

effect. On day 5, one group of rats was pretreated with BIT and the other pretreated with vehicle. The following day, when mu opiate receptors were hypothesized to no longer by functional, animals were tested to determine whether morphine's effects on locomotion were blocked.

8. Highly Selective Opiate Receptor Ligand Accumulates in Brain after Intravenous Administration to Live Animals

The evaluation of the role of opiate receptors in normal brain function and in brain pathology in humans using positron emission tomography (PET) is heavily dependent on the synthesis and development of highly selective radioactive tracers that can be administered to humans with few or no toxic side-effects. Dr. Kenner Rice has recently developed a stable, fluoridated, high affinity opiate receptor antagonist that has been shown to accumulate and label opiate receptors in the baboon using PET. This compound, cyclo-foxy, has also been labeled with tritium and is amenable to *in vivo* analysis in rodent models. In order to determine whether this new compound, ^{3}H-cyclo-foxy, labels the well-described naloxone-sensitive opiate receptor, *in vivo* and *in vitro* autoradiographic experiments were conducted in collaboration with Dr. Candace Pert.

Rats were injected, intravenously, with 30 μCi of ^{3}H-cyclo-foxy and decapitated at 30, 60, and 120 min. following the injection. Dissected brain regions (thalamus, striatum, cerebellum) were processed according to standard binding procedures to determine brain to cerebellar (i.e., specific to non-specific) binding ratios. Sections of each brain were also mounted onto slides and processed for film autoradiographic analysis of binding to opiate receptors. Film autoradiographs were subsequently compared to autoradiographs of ^{3}H-naloxone binding in brain. Additional brain sections from each animal were processed *in vitro* to determine the similarity between *in vivo* and *in vitro* binding densities, the extent of nonspecific binding, and to compare cyclo-foxy binding with that of naloxone.

9. Sex Differences in Brain Opiate Receptors

Endogenous opioid systems have been implicated in the tonic regulation of the hypothalamic-pituitary gonadal axis. Pharmacological studies have shown that administering opioid agonists or antagonists to many species, including man, interferes with reproductive behavior and disrupts gonadal steroid regulation of hypothalamic and pituitary hormones. There is some indirect evidence to suggest that the effects of exogenous opioids are more pronounced in males. In order to explore the possibility that sex differences in opiate receptors might account for some of the different effects of opiates in males and females and to determine the extent of involvement of opiate receptors in reproductive cyclicity, autoradiographic experiments were conducted assessing regional binding densities and binding patterns of ^{3}H-naloxone and ^{3}H-D-Ala-D-Leu-enkephalin in male and female hamsters.

Preliminary experiments indicated that the binding conditions previously established to optimize opiate receptor visualization in rats yielded high (greater than 90%) specific binding in hamster brain tissue, although there were some regional differences between rat and hamster binding patterns. Hamsters were selected for study since they showed extensive ^{3}H-naloxone binding in the hypothalamus, demonstrate marked sexually dimorphic behaviors, have brain sizes that do not differ between males and females; the males have a

well-differentiated sexually-dimorphic nucleus, and females demonstrate rigid, hormone-dependent four-day estrous cycles.

Males, estrous females (peri-ovulatory) and diestrous females were sacrificed and their brains sliced, mounted onto slides, and incubated in vitro with ^{3}H-naloxone while adjacent brain sections were incubated with ^{3}H-DADLE. Slides were apposed to tritium-sensitive film, and autoradiographs analyzed for differences in opiate receptor binding patterns and optical density ratios (optical density of brain region compared to white matter in the same section).

10. Does Reproductive Behavior Increase the Release of Brain Opioid Peptides?

In vivo autoradiography is a procedure developed in this laboratory as a means of monitoring endogenous opioid peptide release by measuring the extent of exclusion of ^{3}H-diprenorphine from opiate receptors after behavioral manipulations. Thus far, this technique has proven successful in showing that three behavioral manipulations that induce naloxone-sensitive analgesia in rats (intermittent foot-shock, cold water swim, and electrical stimulation of the arcuate nucleus) also lead to decreases in ^{3}H-diprenorphine binding in discrete brain regions, presumably because of an increase in receptor occupancy by endogenous peptides. To date, physiologically relevant stimuli that an animal might encounter on a routine basis have not been tested in this paradigm. Since mating has previously been reported to produce an 86-fold increase in plasma beta-endorphin in hamsters, and since the H-P-G axis is thought to be tonically regulated by endogenous opioids, we tested whether copulation might result, too, in an increase in receptor occupancy and consequently exclude ^{3}H-diprenorphine from opiate receptors in brain.

Three groups of male hamsters were tested: one group was mated to 5 ejaculations, injected through an indwelling jugular catheter with ^{3}H-diprenorphine, and subsequently allowed to continue mating for an additional 20 minutes. A second group of animals was injected without any experimental manipulation at matched time intervals, while a third group was injected with the labeled compound at matched time intervals after being exposed to a receptive female that had a mask covering the perineal region, thus eliminating the possibility of mating. Brains were processed according to the methods described for in vivo autoradiography.

11. Effects of Non-opioid Peptides on Brain-Stimulation Escape Thresholds

Several non-opioid peptides, including neurotensin, calcitonin, and vasoactive intestinal peptide, have been shown to possess antinociceptive activity after intracerebral administration. In rats, this activity has been defined by the ability of these endogenous substances to prolong reaction times of simple escape maneuvers to peripheral noxious thermal stimuli on classical analgesic screening assays, such as the hot-plate and tail-flick assays. We are presently investigating the effects of these peptides on escape behavior maintained by aversive electrical brain stimulation delivered to the mesencephalic reticular formation.

In a discrete trial paradigm, rats are trained to press a level in order to escape from the non-contingent onset of the electrical stimulus. Current intensity is varied according to a modification of the psychophysical method of limits in order to obtain thresholds for escape. The major dependent variable is the difference between thresholds obtained before and after drug treatment.

Since each subject serves as its own control, the significance of threshold changes after drug administration is determined by comparing drug-induced changes to those obtained on vehicle control days. In this procedure, alterations in latency to respond afford a measure of non-specific drug effects which might impair the subject's ability to respond.

Previous data have shown that this method is capable of detecting the specific antinocisponsive effects of systemically administered opioids. Thus, morphine, heroin, cyclazocine, pentazocine, as well as ethylketocyclazocine, produce elevations of escape thresholds without altering latency to respond, and at doses which are comparable to those which are active on classical analgesic assays. In addition, the ability of naloxone to lower escape thresholds indicates that there may be a tonic involvement of some endogenous opioid neural substrate in this behavior. However, the minimal effective doses of naloxone are consistently at 8 mg/kg (s.c.). That relatively high doses of this narcotic antagonist are required to produce a significant effect suggests both that whatever opioid component may be involved is not especially sensitive to this prototypical antagonist, and that other endogenous substances probably mediate, in part, the aversiveness of brain stimulation. This latter suggestion provides a rationale for investigating the effects of non-opioid endogenous peptides on brain-stimulation escape behavior in this paradigm. Further, since the utilization of central aversive stimuli bypasses the primary sensory pathways of nociceptive input, it is likely that our observations will reflect the supraspinal processing of aversive stimuli and, possibly, the central underpinnings of negative reinforcement.

Our initial experiment was designed to compare the effects of intraventricularly administered neurotensin and morphine. We have previously shown that neurotensin has antinocisponsive activity on the hot-plate and tail-flick assays. Antagonism of the expected antinocisponsive activities of each drug in the present study is to be tested by concurrent administration of either naloxone or thyrotropin-releasing hormone (TRH). The former antagonist would be expected to mitigate the effects of morphine, but probably not hat of neurotensin. On the other hand, the reported antagonism of various effects of neurotensin by TRH suggested that the latter peptide might antagonize the expected threshold elevation by neurotensin.

12. Use of in vivo Autoradiographic Procedures to Evaluate the Functional Activity of Endorphin Systems Following Brain Stimulation

The arcuate nucleus of the hypothalamus is the major source of beta-endorphin efferents in the CNS, suggesting that it may be involved in pain control. We have tested this nucleus for induction of stimulation-induced analgesia, and subsequently monitored the same animals for the release of opiate peptides, using the technique of *in vivo* autoradiography.

Eight male Sprague-Dawley rats were implanted with bipolar electrodes into the arcuate nucleus. Following recovery, the rats were stimulated in the arcuate nucleus for 1 minute with 100 msec 3/sec bursts of square wave pulses (50 Hz, 50 μsec pulse width, 100 μsec delay). Pain sensitivity was quantified using a hot-plate test (latency to lick rear paw with plate at 55 C).

Following analgesia testing, all of the rats were implanted with intravenous jugular catheters for the autoradiography procedure. Electrical stimulation was

applied to half of them for one minute, after which each rat received an injection of ^{3}H-diprenorphine (50 μCi/kg) while the stimulation continued. After twenty minutes (to allow washout from nonspecific binding sites), the rats were decapitated and the brains frozen intact for slicing. The other four rats were prepared identically, but without any stimulation. Each stimulated rat was matched with a control rat on the basis of cerebellar binding levels. Comparisons were made between anatomically equivalent sections from these matched pairs, which were exposed on the same sheet of ^{3}H-Ultrofilm. Analysis of the film was by computer-assisted densitometry.

Kindling is a process in which repeated intermittent subthreshold stimulation eventually produces a suprathreshold electrophysiological (and behavioral) convulsive response. Kindled convulsive episodes are followed by a post-ictal period of behavioral depression, the duration and severity of which can be attenuated by prior treatment with the opiate antagonist naloxone. This and other lines of evidence suggest that opioid peptides are released centrally during the ictal process and may play a protective role against the immediate repetition of the event. We have employed an in vivo autoradiographic technique which allows the visualization of opioid peptide release in the intact behaving rat in order to study the pattern of opiate involvement in the ictal and post-ictal state.

Four adult male Sprague-Dawley rats were implanted with bipolar stimulating electrodes aimed at the central amygdaloid nucleus. The rats were kindled to full tonic-clonic (Stage 5) seizures over a period of three months and then maintained seizure-free for a further three months. One kindling test session was given to insure sensitivity to stimulation, followed by one more week seizure free. A full Stage 5 seizure was then induced, which was immediately followed by i.v. injection of the high affinity opiate antagonist ^{3}H-diprenorphine (50 μCI/kg) through a jugular catheter. Following a 20 min. wait to allow for washout of the label from nonspecific sites, the animals were decapitated and the brains frozen intact for slicing. Four unkindled control rats with electrodes implanted in the ventral tegmental nucleus were injected identically and matched to kindled rats on the basis of cerebellar (nonspecific) binding levels. Comparisons were then made between anatomically equivalent sections from these matched pairs, exposed on the same sheet of LKB Ultrofilm. Film analysis was by computer-assisted densitometry.

13. Interactive Effects of Neuropeptides and other Psychoactive Substances with the Mesolimbic and Nigrostriatal Dopamine Systems

Calcitonin evokes potent effects on locomotor behaviors. Little, however, is known regarding the specific sites of action of this peptide in modifying locomotor output. In this study we have visualized calcitonin receptor distribution in the rat forebrain with autoradiographic procedures and then evaluated the effects of this peptide on locomotor behavior following direct injections into calcitonin receptor-rich areas.

For receptor visualization, several 24 μm sections were taken through the rostral part of the brain. These sections were exposed to ^{125}I-salmon calcitonin (sCT) and then processed for autoradiography using tritium-sensitive film.

In subsequent studies, rats were implanted with chronic indwelling cannulae guides aimed for the lateral ventricles, nucleus accumbens or arcuate nucleus.

Following recovery the animals were injected in the ventricles or forebrain regions with varying quantities of sCT and then placed in locomotor activity monitors.

In previous studies we have demonstrated that phencyclidine (PCP) has major interactive effects with the mesolimbic and nigrostriatal dopamine systems. For example, systematically administered PCP produces profound ipsilateral rotational behavior in rats lesioned unilaterally in the MFB with 6-OHDA. This suggested that PCP is activating the intact ascending nigrostriatal pathway. Furthermore, direct injections of PCP into the substantia nigra (SN) were found to elicit rotational behavior contralateral to the injection. This suggested that PCP may be activating the ascending nigrostriatal dopamine system by an action in the SN. Previous research has indicated that the nucleus accumbens may be critically involved in the expansion of rotational behavior in unilaterally lesioned rats. We have also shown that the n. accumbens is a critical focus for PCP-induced increases in locomotor behavior. It is possible that PCP may produce part of its effects on rotational behavior through the n. accumbens.

Several studies were conducted to evaluate all of these possibilities. First, rats were implanted with unilateral cannulae guides aimed for the SN. Some animals also received complete hemisections, either rostral or caudal, to the cannula. Other rats were lesioned rostral to the SN with 6-OHDA. Following recovery all rats were injected in the SN with 10 nmoles of PCP and then tested for rotational output. In a subsequent study, rats were lesioned unilaterally in the MFB with 6-OHDA. Some rats also received bilateral lesions of the n. accumbens with either kainic acid or 6-OHDA. Following recovery, all rats were injected with either amphetamine or PCP systemically and tested for rotational behavior.

B. Major Findings

In rats, loss of nicotinic labeling occurred three days after bilateral FR lesions. Some tritiated nicotine labeling was left from the rostral, intermediate, and lateral subnuclei; BTX labeling was reduced to the lateral subnucleus. Scatchard analysis of tissue membranes from IPN revealed a lesion-induced loss of ^{3}H-nicotine binding site number but not affinity. At late survival times, an upregulation of BTX binding occurred. Thus, the IPN possesses both postsynaptic and presynaptic nicotinic receptors, the latter apparently located on cholinergic terminals afferent to the nucleus.

In rhesus monkey brain, tritiated nicotine labeling was particularly dense in certain thalamic nuclei. Labeling was heaviest in all three anterior nuclei (AM, AV, AD). The medial dorsal, lateral dorsal, lateral posterior nuclei, and pulvinor complex contain moderate labeling. The habenular nuclei, intralaminar nuclei, and midline nuclei were conspicuously lacking in label. Intermediate levels of labeling were found in thalamic reticular nuclei, lateral, and medial geniculate nuclei. Muscarinic receptors were also dense in the anterior nuclei, and levels of labeling in most other thalamic nuclei paralleled those of ^{3}H-nicotine. In cerebral cortex, nicotinic receptors were restricted to layer III and this band was densest in primary sensory areas. Muscarinic receptors were found in layers I through III (upper), and below layer III (lower). The cortical distributions of cholinoceptors suggest a nicotinic role in the

processing of sensory afferents, and a muscarinic role in intracortical processing.

Unilateral 6-hydroxydopamine injections depleted striatal DA almost completely, and led to retrograde degeneration of SNC DA perikarya. Tritiated nicotine labeling in DA terminal and cell body regions was reduced in both the nigrostriatal and mesolimbic systems. The presence of nicotine receptors on DA cell bodies and kinases is consistent with electrophysiological and biochemical findings. Kainic acid, introduced into or near the PPN, produced a rapid-onset and dose-related remote excitation of SNC DA neurons. This finding, consistent with a cholinergic PPN-SNC projection terminating on postsynaptic nicotinic cholinoceptors, is being investigated pharmacologically.

Results showed that destruction of dopamine-containing neurons did not reduce the density or alter the distribution of stereospecific ^{3}H-naloxone binding or ^{3}H-D-Ala^2-D-Leu-enkephalin binding in the substantia nigra pars compacta or the pars reticulata, indicating that the bulk of opiate receptors (mu and delta) are not localized on dopamine cells.

Kainic-acid lesions of the striatum reduced ^{3}H-naloxone binding (optical density measurements) on the lesioned side only when they were placed caudally and involved the globus pallidus. More discrete electrolytic lesions of the globus pallidus and the internal capsule also reduced or completely eliminated ^{3}H-naloxone binding density in both the pars compacta and pars reticulata, while not affecting the dense ^{3}H-naloxone binding in the accessory optic tract which courses just dorsal to the substantia nigra. These results suggest that opiate effects on dopamine function and dopamine-linked behaviors are likely to be mediated by actions at presynaptic receptors localized on nigral afferents.

BIT, by itself, produced a morphine-like stimulation of locomotor activity after acute infusions into the ventral tegmental area. Twenty-four hours later, however, using each animal as its own control, the morphine-induced stimulation of locomotor activity was inhibited by about 50% in BIT pretreated rats at 1 hour after morphine. Baseline locomotor activity was not affected by BIT nor were there any discernible toxic effects of the drug treatment. Vehicle-treated rats continued to respond to morphine with increased locomotion.

This experimental procedure, involving local application of mu- and delta-selective alkylating agents into brain tissue holds promise for assessing the relative contribution of mu and delta receptors in mediating the effects of both centrally- and systemically-administered opiates, and in localizing brain regions involved in narcotic-induced behavioral changes.

The highest brain to cerebellar binding ratios (determined by liquid scintillation counting) after intravenous injections of ^{3}H-cyclo-foxy were obtained from homogenates, supernatants, and from dissected brain regions scraped from microscope slides at 60 min. An additional experiment, in which rats were injected with 10, 30, 100, or 300 μCi of ^{3}H-cyclo-foxy confirmed that at 60 min. excellent retention of label was found with 30 μCi in opiate receptor-dense regions (i.e., striatal patches, subcallosal streak, thalamus) with negligible labeling of non-opiate receptor-dense areas (corpus callosum, cerebellum).

Pattern analysis of autoradiographs showed that the binding of ^{3}H-cyclo-foxy was virtually identical in vivo and under in vitro binding conditions. Additionally, brain sections that had previously bound ^{3}H-cyclo-foxy in vivo could be washed free of the label in vitro and were shown to rebind both ^{3}H-cyclo-foxy or ^{3}H-naloxone in the same patterns. Binding of ^{3}H-cyclo-foxy in vitro was completely inhibited by incubating brain sections with unlabeled naloxone. These data confirm that the new compound, cyclo-foxy, binds to the well-described, naloxone-sensitive opiate receptor in vivo and in vitro and suggests that it will be an excellent candidate for use in PET scanning in live animals and humans.

Of the 25 brain regions sampled, two showed sex differences in ^{3}H-naloxone binding. The medial-preoptic-anterior hypothalamic region of the male bound naloxone in a U-shaped pattern, while the same region bound naloxone in the female in a V-shaped pattern. Quantitative analyses confirmed that the sexually dimorphic nucleus of the male showed denser binding than the sexually dimorphic nucleus of the estrous female. Likewise, the stria terminalis in the male and in the diestrous female bound ^{3}H-naloxone more densely than in the estrous female. No differences were found among groups for ^{3}H-naloxone binding in white matter and density measurements for the left and right sides of the brain were highly correlated (greater than .96).

In contrast to ^{3}H-naloxone binding, ^{3}H-DADLE binding to delta receptors was extremely sparse in the hypothalamus of both sexes and no sex differences were found in any region. These data confirm that two brain sites thought to participate in the regulation of the H-P-G axis showed sex-linked differences in opiate receptors. Moreover, since binding densities of diestrous females were more similar to densities of males than estrous females (particularly in the stria terminalis) these data suggest that endocrine status of the adult animal may modulate opiate receptors.

In order to determine whether these sex differences might be attributable to differential occupation of receptors by endogenous ligand at the time of sacrifice, to differences in receptor affinity for the labeled ligands, or to differences in opiate receptor numbers, additional experiments were performed on estrous and diestrous females. Extensive prewashing of tissues before incubation with ^{3}H-naloxone does not alter the binding density in estrous and diestrous females, suggesting that the differences across the estrous cycle are not due to endogenous opioid occupation of receptors. Incubation of brain slices with a ten-times greater concentration of ^{3}H-naloxone resulted again in differences between estrous and diestrous females, indicating that differences in binding density are likely to be due to an increased number of opiate receptors in diestrous females rather than a change in the affinity of the receptors for naloxone.

These results indicate that mu receptors, but not delta receptors may be modulated by endocrine status in the female hamster and change their numbers with the reproductive cycle. To explore the possibility that steroid hormones may participate in regulating the numbers of receptors in the hypothalamus, experiments are currently in progress to ascertain the effects of gonadectomy, with or without steroid hormone replacement, on opiate receptor binding in females.

While quantitative analysis has not been completed, there are discernible differences in ^{3}H-diprenorphine binding densities between the control males and the mated males in the medial basal hypothalamus, ventromedial hypothalamus, preoptic area, substantia nigra, and medial habenula and stria terminalis. Both liquid scintillation counting and autoradiography indicate that the mated males show reduced binding when compared to non-exposed control males. Since binding density appears to be reduced in the males exposed to estrous females but not permitted to mate, additional experiments will be conducted to assess the possibility that exposure to a receptive female under conditions previously associated with mating behavior may be sufficient to induce increased opiate receptor occupancy by endogenous opioid peptides.

We have found that doses of morphine (5-15 nmoles) significantly and specifically elevated escape thresholds, as expected. In addition, this effect is antagonized by naloxone (4 mg/kg, i.p.) administered immediately prior to intraventricular injection. In contrast and unexpectedly, neurotensin over the same dose range produces significant decreases in escape thresholds. It is clear that this effect of neurotensin is unlike those previously observed by others, as well as by us, utilizing other assays sensitive to antinociceptive drugs. Presently, we are trying to characterize this effect of neurotensin by testing the interactions between neurotensin and morphine, naloxone, as well as TRH. These forthcoming data might suggest whether the underlying neurotensin and opioid neural effects reflected in the observed behavior represent a functional antagonist of the systems activated by each drug.

Preliminary data suggest that salmon calcitonin, on the other hand, may indeed produce an antinocisponsive effect at doses as low as 0.5 nmole in this paradigm. However, further verification and characterization of this effect are required. Previous work in this laboratory employing shuttle-box running behavior has demonstrated that morphine increases the latency to terminate aversive brain stimulation. We are presently engaged in assessing the effects of representative opioid peptides to attenuate aversive midbrain electrical stimulation. In order to completely characterize this behavior, we will measure the effects of several peptides on the latency to respond over a range of current intensities.

Since there is some controversy concerning the relative involvement of various opioid receptor subtypes in the antinociceptive efficacy of this class of drugs, particularly at supraspinal sites, the peptides will represent a spectrum of relative selectivities for mu-, kappa-, epsilon-, and delta-opioid subtypes. These peptides will include beta-endorphin, dynorphin A (1-13), and several protected enkephalin analogues.

Electrical stimulation of the arcuate nucleus caused a significant increase in hot-plate latencies which lasted beyond the period of stimulation. Threshold current for inducing analgesia was 600 μAmp, with maximal effect at 1-1.2 mAmp. Pretreatment with naloxone (5 mg/kg i.p. 15 min. before testing) had no effect on baseline latency, but blocked the increase due to stimulation. These arcuate-implanted rats differed from a similarly treated group with electrodes in the periaqueductal grey in which analgesia did not outlast the stimulation and was not naloxone-reversible.

Significant bilateral decreases (up to 22%) in diprenorphine binding were found in a number of brain regions following arcuate nucleus stimulation, including the anterior and medial amygdala, striatal fundus, bed nucleus, preoptic area, hypothalamus, and periaqueductal grey. No changes were found in basolateral amygdala, medial thalamus, cortex, or white matter. This pattern of changes indicates increased receptor occupancy in terminal projection areas of the arcuate nucleus due to stimulation-induced release of beta-endorphin.

In the kindled group, acute seizure induced significant bilateral decreases in diprenorphine binding at numerous levels of the forebrain. These included 35-40% decreases in specific binding in cingulate cortex, nucleus accumbens, and dorsal hippocampus, while no significant change was seen in the caudate or motor cortex. At more posterior levels, binding in the basolateral amygdala was decreased by 48%, while smaller decreases (20-30%) were found in the ventral hippocampus, cortical amygdaloid nucleus, hypothalamus and sensory cortex. No change was seen in the thalamus or in any more posterior structures. These results suggest widespread occupation of forebrain opioid receptors by endogenous opiates released during the ictal process, which prevented the binding of the exogenous radio-labeled opiate ligand. Alternatively, kindling may have induced region-specific changes in opiate receptor binding characteristics.

Autoradiographic analyses revealed exceptionally heavy binding of sCT in the nucleus accumbens. The caudal portion of the nucleus appeared to have somewhat higher labeling than the rostral segment. Little binding was evident in the caudate nucleus or other forebrain regions. Lesions of the ascending dopamine pathway with 6-OHDA failed to alter sCT binding in the n. accumbens. Injections of kainic acid into the n. accumbens, on the other hand, decreased binding on the lesioned side. These findings suggest that sCT binding sites are located on cell bodies intrinsic to the n. accumbens and not on DA terminals that innervate this region.

Intraventricular injections of sCT (1, 10, or 25 nmoles) produced a dose-dependent long-lasting decrease in locomotor behavior. Bilateral injections of sCT into the n. accumbens resulted in an even more dramatic response. As little as 0.02 nmoles decreased horizontal locomotor activity by 53% in the first 15 min. Higher doses (0.1-2.0 nmoles) had an even greater effect. Injections of 0.5 nmoles into the caudate nucleus, on the other hand, had little effect on locomotor output.

These findings indicate that sCT is an extremely potent modulator of locomotor output. The effect appears to be mediated predominantly through the interaction of this peptide with sCT binding sites on neurons intrinsic to the n. accumbens.

Complete brain hemisections rostral to the SN failed to prevent rotational behavior following intranigral PCP injections. 6-OHDA lesions were also ineffective. Hemisections caudal to the SN, on the other hand, significantly attenuated this effect of PCP. These findings indicate that the effects of PCP in the SN are expressed through pathways that project caudally and not through the ascending nigrostriatal system. 6-OHDA lesions of the n. accumbens failed to alter rotational behavior induced by PCP in rats lesioned immediately in the MFB. Kainic acid lesions, on the other hand, were effective in preventing this effect of PCP. These findings suggest that PCP induces rotational behavior in

MFB-lesioned rats through an action in the n. accumbens which is independent of dopamine.

Significance to Biomedical Research and the Program of the Institute

Since opiate alkaloids produce some of the most profound behavioral and physiological effects, endogenous opiates (which are mimicked by the alkaloids) must serve an important role in regulating emotions as well as physiological and sensory processes. The use of our newly developed autoradiographic procedures, which allow a measurement of ongoing activity in these systems, will hopefully reveal the functional significance of opiate pathways in brain. Since nicotine is one of the most abused substances in society, understanding its neuronal mechanisms of action will aid in understanding the abuse properties of this substance. It has been suggested that the cholinergic system also plays an important role in mental disorders. It is therefore necessary to understand the functions of this system in brain and to analyze its interactive effects with other neurotransmitter systems such as dopamine. Phencyclidine is also an increasingly abused substance. Furthermore, phencyclidine produces effects in man very similar to some of the primary symptoms of schizophrenia. For these reasons, it is valuable to understand the mechanisms of action of this class of compounds. Besides endorphins, the brain contains numerous other peptides which undoubtedly serve important regulatory functions. It is important to identify the distribution of binding sites for other substances in brain and to analyze their physiological and behavioral effects with micro-injection mapping techniques. Alterations in the activity of these systems may underlie a number of neurological and psychiatric disorders.

Proposed Course of Project

1. A heavy emphasis will be placed on developing techniques to measure the *in vivo* binding of various neuropeptides. This will allow us to assess the functional activity of numerous peptide systems in brain.

2. Somatostatin binding will be mapped autoradiographically in brain. Attempts will be made to assess the functional effects of somatostatin in areas of high binding. Studies will also be undertaken to ascertain the association of somatostatin receptors with various neurotransmitter systems.

3. The *in vivo* autoradiographic technique will continue to be used to define endorphinergic circuitry activated by various behavioral and physiological manipulations.

4. The brain circuitry underlying aversive brain stimulation will be analyzed using 2-D6 methodology. The effects of various peptides will be examined on aversive brain stimulation thresholds.

5. We will continue to examine the physiological and behavioral effects of neuropeptides.

6. 2-D6 methodology will be used to assess the functional activity of brain circuitry during specific behaviors in rats.

7. Attempts will be made to define the neurochemical events associated with uncontrolled versus controlled shock.

Publications

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Fabbri, A., Gnessi, L., Maretti, C., Pert, C., Pert, A., and Fraioli, F.: The calcitonin neuropeptide: clinical and experimental studies. In Pecile, A. (Ed.): Calcitonin. Amsterdam, Elsevier/North Holland, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00400-03 BP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Phosphorylation in Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jitendra Patel, Unit on Neurochemistry, BPB, NIMH

Paul J. Marangos, Chief, Unit on Neurochemistry, BPB, NIMH; Dr. Robert M. Post, Chief, Biological Psychiatry Branch, NIMH; Susan Weiss, Biologist, Biological Psychiatry, NIMH; Douglas Kligman, Staff Fellow, IR/BG, NHBLI; Robert V. Rebois, Staff Fellow, IR/P, NINCDS; Thomas L. O'Donahue, Staff Fellow, NINCDS; Dr. Ehud Klein, Unit on Anxiety and Affective Disorders, Biological Psychiatry Branch, NIMH

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, NIMH; National Heart, Lung and Blood Institute; National Institute of Neurological and Communicative Disorders and Stroke

LAB/BRANCH

Biological Psychiatry Branch

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Unit on Neurochemistry

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We had previously described a unique 87 kilodalton protein of which the phosphorylation was inhibited by calcium binding proteins, S-100 and calmodulin. Further evidence was obtained to establish that this protein represented a major substrate of calcium/phospholipid dependent protein kinase (protein kinase C). The mechanisms of inhibition of the 87k protein by S-100 and calmodulin was addressed.

Effects of various neuropeptides on the phosphorylation of synaptic membrane proteins was investigated. It was found that calcitonin and somatostatin are potent inhibitors of protein phosphorylation. Detailed characterization of this effect of these neuropeptides was performed.

The involvement of protein kinase C in the modulation of receptor sensitivity was further investigated using human choriogonadotropin (hCG) sensitive adenylate cyclase in cultured leydig cells as a model system.

The effect of chronic treatment of rats with lithium on the CNS protein phosphorylation system was investigated. Lithium treatment was found to produce a marked increase in the phosphorylation of a 64 kilodalton protein.

Project Description:

Objectives: Considerable evidence now indicates that cyclic AMP along with calcium represents the main second messengers of the CNS. In this regard these agents play a pivotal role in mediating various actions of neurotransmitters and other neurohumoural agents. Actions of the second messengers are predominantly mediated via certain protein kinases that catalyse the phosphorylation of specific proteins.

Briefly, the second messenger system in the CNS involves the following components. Cyclic AMP is produced in response to various agonists by adenylate cyclase and mediates its action via cyclic AMP-dependent protein kinase (aPK). Intracellular calcium levels are also under the regulation of various cell surface receptors by a mechanism that is often referred to as "PI turnover system". Under this system agonist-induced receptor activation leads to a stimulation of a specific phosphodiesterase which is capable of hydrolyzing polyphosphotidylinositide to produce polyphosphoinositide (IP3) and diacyglycerol (DAG). IP3 thus produced alters the permeability of the plasma membrane to extracellular calcium, resulting in an intracellular mobilization of calcium. Calcium via calmodulin then activates several enzymes including calmodulin-dependent protein kinase (kPK). Calcium, in concert with DAG and phosphotidylserine, also activates calcium/phosphotidylserine-dependent kinase (cPK).

Since aPK, cPK, and kPK are the main mediators of the second messengers, and therefore the neurotransmitters, they play a key role in neurotransmission. The aim of our studies is to obtain a better understanding of the involvement of these protein kinases in CNS functions. In this task, as before, we have adopted the following approach.

1. Characterization of protein kinase and its substrates.
2. Investigation of possible changes in protein kinase activity and substrates in animal models where change in synaptic efficacy or function is suspected.
3. Identify specific cellular processes under the regulation of protein kinase.

Methods Employed: Gel electrophoresis, autoradiography, enzyme kinetics, protein purification, tissue culture.

Major Findings: We have previously reported that phosphorylation of a major CNS 87 kilodalton phosphoprotein could be inhibited by calcium binding proteins, S-100 and calmodulin. We have since established by several means that this phosphoprotein is a major substrate for the cPK in the CNS. In collaboration with Dr. T. O'Donahue, we have also recently demonstrated that the 87k protein is one of the major substrates for the cPK in intact cell.

In collaboration with Dr. D. Kligman, we were also able to identify a novel heat stable factor purified from bovine brain which apparently inhibited the phosphorylation of the 87k phosphoprotein and also stimulated the phosphorylation of a 90k protein. The phosphorylation of the 90k was not apparent in the absence of this factor. Our initial studies had led us to believe that this heat stable factor also possessed the property of stimulating the growth of neurites in cultured CNS neurons. However, on further more extensive purification we showed that the factor responsible for the stimulation of the 90k protein was devoid of such trophic activity. Our present results indicated the heat stable factor

responsible for stimulating the phosphorylation of the 90k protein is a 37 kilodalton protein. The mechanism by which the 37k protein mediates its effect on protein phosphorylation is at present not clear. Preliminary work using partially purified cPK however indicates that the 37k protein is a highly potent activator of its activity.

Using partially purified cPK we have also investigated the mechanism by which calmodulin and S-100 inhibit the phosphorylation of the 87k protein. Due to the present unavailability of pure 87k phosphoprotein, we have examined cPK activity using histone and myelin basic protein as cPK substrates. Under these conditions it was found that calmodulin and S-100 failed to inhibit cPK activity. The result of such studies have, however, proved difficult to interpret as the cPK preparation contained a number of contaminating proteins. An effort to obtain a homogenous preparation of cPK is in progress. Availability of pure cPK will also facilitate the purification of the 87k phosphoprotein.

The effect of neuropeptides on the phosphorylation of synaptic membrane proteins were examined. Of the various peptides examined, calcitonin and somatostatin were found to be potent inhibitors of protein phosphorylation. These neuropeptides produced a marked inhibition of phosphorylation of the 64k and 50k proteins.

Neuropeptide structure-activity relationship for the inhibition of protein phosphorylation was performed. In the case of calcitonin, salmon and eel calcitonin was found to be much more potent than that from human. Activity of various calcitonin analogs suggested a whole calcitonin molecule was required for the inhibitory activity, and an alteration even of a single amino acid produced a marked decrease in the potency of that peptide to inhibit phosphorylation. Also, the structural requirement for the inhibition of protein phosphorylation was consistent with that observed with some of the behavioral effects of calcitonin. It is interesting to suggest that the inhibition of protein phosphorylation by calcitonin may be a mechanism by which it mediates some of its CNS actions. It is also possible that some of the effects of somatostatin are similarly mediated. In this regard it is noteworthy that a number of investigators have postulated an intracellular site of action for somatostatin.

We have recently examined the identity of the 64k and the 50k phosphoprotein. It was found that these proteins bind to calmodulin. This and other observations have lead us to believe that the 64k and 50k proteins respectively represent the autophosphorylated alpha and beta subunits of calmodulin-dependent protein kinase.

The following work was performed with Dr. Ehud Klein. It has been widely suggested that lithium may mediate its clinical effects by inhibiting the complete breakdown of IP3. A possible consequence of this effect of lithium would be a perturbation of the protein kinase system of the CNS. We undertook to experimentally examine this possibility. Rats were fed on a diet containing lithium salt for a period of two weeks, and the phosphorylation of various CNS proteins was examined. Increased phosphorylation of 64k phosphoprotein was obtained in the rats exposed to lithium.

The effect of lithium on the 64k protein appears to be widespread and was noted in all the brain areas examined (cortex, caudate, hippocampus and cerebellum). The 64k phosphoprotein is present in the brain, both in the particulate and cytosolic

fractions. However, the lithium-induced enhanced phosphorylation of this protein could only be observed in the particulate fraction. Also, the lithium effect was only observed when the phosphorylation was performed under basal conditions; addition of calcium to the phosphorylation assay which causes a marked increase in the phosphorylation of the 64k protein, abolished the effect of lithium. It is very likely that the 64k phosphoprotein represents the alpha subunit of calmodulin-dependent protein kinase. Experiments to establish this are in progress.

Previous work, performed in collaboration with Dr. Robert Rebois, strongly demonstrated that the activity of the gonadotropin-responsive adenylate cyclase system could be modulated by cPK. The focus of our current studies is to identify the specific phosphoproteins responsible for this action of the cPK. We have recently demonstrated that the addition of partially purified cPK from bovine brain to plasma membranes isolated from leydig cells results in the phosphorylation of a number of membrane proteins and the desensitization the adenylate cyclase. The ability to modulate the cyclase activity in a cell-free system will considerably facilitate our elucidation of the cPK-mediated modulation of adenylate cyclase.

Significance of Biomedical Research and the Program of the Institute: The study of protein phosphorylation is of vital importance in the quest for better understanding how the neurotransmitters mediate their action. Such an understanding is the necessary prerequisite for the appreciation of the biological basis of normal and abnormal behavior.

Proposed Course of the Project: These studies are expected to continue for the next several years.

Publications

Patel, J., Fabbri, A., Pert, C., Gnessi, L., Fraioli, F. and McDevitt, R.: Calcitonin inhibits the phosphorylation of various proteins in rat brain synaptic membranes. Biochem. Biophy. Res. Commun., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01831-09 BP

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic and Clinical Studies of Neuronal and Glial Enolases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul J. Marangos, Ph.D., Chief, Unit on Neurochemistry, BPB, NIMH

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A.G.E. Pearse	Prof. Emeritus	Royal Med. School, London
S.R. Bloom	Professor	Royal Med. School, London
J. Minna	Chief, Med. Oncology Branch	NCI/NNMC
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COOPERATING UNITS *(if any)*

Royal Med. School, London; NCI/NNMC; Texas U.; Vanderbilt Med. Sch.; Va. U; Md. U.; LN, NIA; Loyola U., Chicago; Mich U., UCLA Med. Sch.; Children's Hosp., Phila.; Div. of Psychiatry, MRC; Div. Nuclear Med., Johns Hopkins; Finsen Inst., Denmark

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.5	0.9

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies involving neuron specific enolase (NSE) have for the most part centered on clinical applications. We have further documented and characterized the utilization of serum NSE levels and NSE immunocytochemistry as diagnostic and prognostic tools in various neuroendocrine neoplasms. Specifically, we have further evaluated the effectiveness of monitoring the clinical course of both oat cell lung cancer and pediatric neuroblastoma utilizing our serum NSE radioimmunoassay. At the basic level, studies are currently being initiated to develop a specific suicide inhibitor for NSE that would have the potential to be labeled with positron emitters and used in diagnostic brain imaging. Our efforts are also continuing concerning the cloning of the NSE gene, which will greatly facilitate our studies of the differentiation of neurons. It is likely we will obtain the cDNA probe during the next year and perform some in situ hybridization studies. Since NSE is a marker for neuronal differentiation, the elucidation of factors which control its synthesis should provide basic insights concerning the molecular mechanisms involved in neural maturation.

PHS 6040 (Rev. 1/84) GPO 904-917

Other Professional Personnel

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D. Johnson	Oncologist, Assoc. Prof.	Vanderbilt Med. School
A. Greco	Oncologist, Assoc. Prof.	Vanderbilt Med. School
M. Brownstein	Chief, Lab. of Cell Biology	NIMH
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R. Prinz	Endocrinologist	Loyola Univ., Chicago
R. Lloyd	Endocrine Surgeon	U. of Mich., Ann Arbor
R. Seeger	Pediatric Oncologist	UCLA Medical School
A. Evans	Chief, Pediatric Oncology	Children's Hosp., Phila.
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V. Balasubramanian	Asst. Professor	Johns Hopkins, Div. of Nuclear Medicine
A. Pedersen	Clinical Oncologist	Finsen Inst., Copenhagen
F. McDowell	Oncologist	University of Maryland
B. Trump	Toxicologist	University of Maryland

I. Project Description

A. Objectives

The study of proteins either unique to or particularly important to brain function is a theme common to all of the work within the Unit on Neurochemistry. The three major areas of concentration for the past nine years have been neuronal and glial proteins, brain neurotransmitter and neuromodulator receptor proteins, and, for the past three years, protein kinases and protein phosphorylation mechanisms. Our work on neuronal and glial proteins, specifically, neuron specific enolase (NSE), non-neuronal enolase (NNE), and the S-100 Protein, were originally rather unique to our group since, when we started, only a handful of groups were involved in such studies.

Our motivation for studying proteins specifically localized to neurons centers on the assumption that such proteins will subserve specific neuronal functions and that their functional characterization will provide insights regarding neural function. In the case of NSE, we have shown that this protein is involved in neural energy metabolism (i.e., it is an enolase), that it is structurally and immunologically totally distinct from other enolases, and that it is exclusively localized in neurons and neuroendocrine cells. We have also shown that NSE only appears in neurons that have formed functional synaptic contracts so that it is a valuable marker for neuronal differentiation. We have, within the past five years, applied the NSE methodology to clinical problems, using our radioimmunoassay and immunocytochemistry staining methodologies. The NSE methodology we have developed has proven to be of great value both diagnostically and to follow the clinical course of several neuroendocrine neoplasms.

Our major objectives during the past year have been to clinically further exploit the NSE methodology concerning neuroendocrine neoplasms and degenerative neurologic diseases. At the basic level, we are trying to learn more about the specific function of NSE and what regulates the switch during neuronal differentiation. Along these lines, we are attempting to obtain the cDNA for NSE and to study, at the genetic level, the factors that regulate expression of this antigen.

We have previously shown that NSE serum levels are elevated in greater than 80% of small cell lung cancer patients. This neuroendocrine tumor contains high levels of NSE, and serum levels in patients often become elevated to 100-fold higher than normal. Serum NSE levels follow the clinical course of the illness in that they decrease to normal during chemotherapy-induced remission and return to elevated levels during relapse. This has made the NSE serum assay a very important component of patient monitoring.

B. Methods Employed

Radioimmunoassay, immunocytochemistry, isoelectric focusing, clinical procedures such as surgery, blood drawing, etc.

C. Major Findings

Recent studies, done in collaboration with Drs. Johnson and Greco at Vanderbilt Medical School, have shown in small cell lung cancer patients with elevated serum NSE levels, that remission can be seen much earlier using the NSE serum assay when compared to conventional means of diagnosis (chest x-ray). These results are very exciting, since they indicate that salvage chemotherapy can be

started substantially earlier and possibly affect the survival time. These studies have been published in Cancer Research.

Histochemical studies have also progressed during the past year, showing that dense core granulated tumors of the lung can be easily marked by their ability to stain with our specific antihuman NSE serum. These studies, done in collaboration with Drs. McDowell and Trump at the University of Maryland, indicate that the identification of these rather heterogeneous tumors can now be greatly simplified with a consequent improvement of patient management. These studies are in press in Arch. Pathol. Lab. Med.

Our long and productive collaboration with Dr. Julia Polak in London has continued this past year, with further immunocytochemical studies revealing that NSE is a valuable aid in tumor typing both by itself and in conjunction with other markers. This has been shown to be true in retinoblastoma, with the studies being published in Virchows Arch. [A], and in neuroendocrine tumors of the lung, with these studies being published in J. Pathol. We have also employed the strategy of using multiple markers to visualize the neuroendocrine cell system in the periphery in non-transformed tissue. Combined immunostaining with NSE, glial fibrillary acidic protein (GFAP) and S-100 antiserum have been shown to be particularly powerful in this regard with both neural and glial elements of the peripheral nervous system and neuroendocrine system being clearly visualized. This enables one to clearly define the cellular networks that constitute peripheral tissue innervation and, more importantly, to visualize with a high degree of precision the neuroendocrine cellular networks in endocrine glands and organs. These studies have been published in Histochemistry. Studies done using antisera to both NSE and the neurofilament proteins have also proven to be very useful in marking and visualizing the intrinsic innervation of peripheral organs and glands. This work is currently in press in Histochemistry. The multiple marker approach has been shown to identify, with a high degree of accuracy, virtually all tumors of neuroblastic origin and greatly simplify the task of the pathologist in identifying tumors. The studies which exemplify the value of this approach are currently in press in Virchows Arch. [A].

All of the studies resulting from our collaboration with Dr. Polak clearly show that NSE provides the key to identifying the peripheral neuroendocrine system. This system has been termed the APUD (Amine precursor uptake and decarboxylation) system, and has been postulated to be composed of neuron-like or paraneuronal cells. Our data showing that NSE (a neuronal antigen) is present in all of these peptide-secreting APUD cells has provided substantial support for the hypothesis. NSE immunostaining now represents the easiest and most reliable way of visualizing this important neuroendocrine system, as well as identifying new putative APUD cell types. NSE, therefore, is an easily quantifiable entity that directly links both the nervous and neuroendocrine systems.

This year we have begun a new collaboration with Dr. Anders Pedersen in Copenhagen, who has a large group of oat cell lung cancer patient material (CSF and sera). We have shown that CSF NSE levels are highly elevated in oat cell patients with brain metastases. These studies are being prepared for publication and should prove useful in managing oat cell patients. We have also designed another study, with both Dr. Pedersen and Dr. Johnson at Vanderbilt, in which we will look at serum NSE levels immediately following the initiation of chemotherapy in oat

cell patients. A preliminary and somewhat serendipitous observation made about six months ago showed enormous elevations of serum NSE immediately following (24 hours) the initiation of chemotherapy in two oat cell patients. This led us to speculate that the initial anti-tumor effect of chemotherapy might be reflected by increases in serum NSE. This hypothesis is now being tested with serum samples from both Copenhagen and Vanderbilt. If results prove to be positive, this could markedly affect the treatment course of small cell lung cancer by rapidly identifying an effective drug combination for each patient.

Our second major clinical focus has been in the area of pediatric neuroblastoma, where results we have obtained over the past two years have shown that serum NSE levels are elevated in 98% of these patients. These studies have been done in collaboration with Drs. Zeltzer, Seeger, and Evans. The recently recognized importance of NSE for both prognostic and diagnostic purposes in neuroblastoma patients is perhaps best reflected by the fact that the recently published volume entitled "Recent Advances in Neuroblastoma Research" had four chapters dealing with NSE. Our studies have been extended this year to show that serum NSE levels are determined by the stage of the illness (lowest in Stage I and highest in Stage IV) and that the time of survival can be predicted by the serum NSE level. We have also now increased our N to over 300 patients and repeated our findings with another major patient group (Children's Hospital, Philadelphia). With patients ranging from 0-1 year old, our N is now 24 (last year it was 15), with the same result still holding; i.e., all patients with serum levels below 100 ng/ml survive at five years, whereas those with serum levels over this value die within two years. This result is defining a new neuroblastoma patient group and has the potential of being very useful concerning decisions relating to the aggressiveness of treatment. The studies described above are currently in press in Cancer and in J. Clin. Oncology.

Clinical studies concerning degenerative neurological disorders are still ongoing, with the result so far being disappointing. We have looked at Alzheimer's CSF and sera with only modest changes observed (CSF increases). We are currently changing our approach in this area and are attempting to look at whether such patients have been immunologically sensitized to NSE. Since the degenerative disorders are progressive and occur over years, it is unlikely that raised CSF or serum NSE levels would be measurable, since the kinetics of tissue degeneration are not known. We, therefore, deem it desirable to take advantage of the immune system's natural memory and assess whether or not it is sensitized to NSE in these patients. This approach assumes that elevated serum NSE levels will stimulate the immune system, since normal levels are so low. These studies are currently being planned, and we are trying to assemble the requisite expertise (clinical immunologist so interested) to perform such studies. These approaches (lymphocyte stimulation test and skin test) will also be applied to psychiatric patients and expanded to include CNS antigens other than NSE; i.e., the S-100 Protein. This approach is also being tried on our oat cell lung cancer patients.

Basic studies regarding NSE have centered in several areas, including electron microscopic localization and the cloning of the NSE gene. Studies in the former area have shown that NSE is much lower in myelinated axons compared to unmyelinated axons, and that, in general, higher levels are found in the receptive area of the neuron; i.e., cell body and dendrites. Purkinje cells were also shown to have less NSE than other neuronal cell types. This study also supplied the first direct evidence of differential levels of NSE in various neurons, suggesting a correlation

between NSE level and the functional state of the neuron. These studies have been published in J. Histochem. Cytochem.

With regard to our work concerning the cloning of the NSE gene, these studies have been proceeding rather slowly due to the nature of our collaboration. Studies were begun with Dr. Brownstein, who in turn attempted to get our pure NSE sequenced at the Salk Institute. This did not happen as planned. We have since learned that another group has obtained the cDNA probe for NSE and we will now attempt to obtain this from them. Our goal in these studies is to characterize the regulation of NSE synthesis and the mechanism of the NNE to NSE switch that we described some six years ago. Upon obtaining the probe, we will do in situ hybridization studies in an effort to determine when various cells make the mRNA necessary for NSE synthesis. We are hopeful that some results will be generated in the coming year on this very important project.

Another important project that has been started this year relates to the development of specific suicide inhibitors for NSE. These studies are being done collaboratively with Dr. Balasubramanian at Johns Hopkins. Here, our goal is to develop a substrate analogue for NSE that will bind specifically to it. Given the structural difference between NSE and other enolases, this should be possible. If we are successful, it will be possible to label this compound with fluorine 18 or other positron emitting atoms. This may provide a new brain imaging technique that will have the key advantage of being neuron specific. These studies, if successful, have the potential of developing a new clinical tool that will be useful in both neurological and psychiatric patients.

D. Significance to Biomedical Research and the Program of the Institute

The NSE methodology has turned out to be a major new technique for probing nervous tissue anatomy and physiology. At the basic level, NSE immunostaining serves as the current best marker for neurons and neuroendocrine cells, as well as a unique marker for neural differentiation. Clinically, we have shown that serum levels of NSE are of both diagnostic and prognostic value in two major neuroendocrine neoplasms; i.e., oat cell lung cancer and neuroblastoma. The development of the NSE methodology at both the basic and clinical levels represents a graphic demonstration of how the Institute's long-range funding support can be successfully applied.

E. Proposed Course of the Project

The project is projected to continue for several years.

Publications

Vinores, S.A., Herman, M.M., Rubinstein, L.J., and Marangos, P.J.: Electron microscopic localization of neuron specific enolase in rat and mouse brain. J. Histochem. Cytochem. 32: 1295-1302, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01833-05 BP

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adenosine Receptors in the CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul J. Marangos, Ph.D.	Chief, Unit on Neurochemistry, BPB, NIMH	
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R. M. Post	Chief	BPB NIMH
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J. Daly	Chief	LBC, NIADD

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.6	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past year, our studies concerning the adenosine receptor, the adenosine uptake site and the "peripheral type" and central type benzodiazepine receptors have continued to focus on the possible mechanisms whereby these neural systems modulate neuronal activity. We have succeeded in solubilizing both the adenosine receptor and uptake site and show that they remain distinct from one another. We have further characterized the interaction between calcium antagonists and the adenosine system and have completed a series of studies showing that chronic carbamazepine administration leads to an upregulation of adenosine receptors in the brain. The adenosine uptake site has been kinetically characterized in brain from five different species, showing that dipyridamole is a very poor inhibitor of nitrobenzylthioinosine binding in rat brain compared to guinea pig and human brain. Studies done in Maudsley reactive rats have shown no changes in brain benzodiazepine binding sites but have, for the first time, shown region specific alterations in adenosine receptors. We have also completed studies during the past year that have shown that the "peripheral type" benzodiazepine binding site is upregulated following chronic ethanol administration to rats and that this increase in the number of binding sites persists during ethanol withdrawal. Increases in brain [^{3}H]Ro5-4864 binding sites are, therefore, associated with both dependence and withdrawal. The autoradiographic localization of adenosine uptake sites has been compared directly to that of adenosine receptors in rat brain, showing that the two sites are differentially distributed. High levels of both sites appear in the caudate, thalamus and superior colliculus, suggesting that adenosinergic neurons might be present in these brain regions.

Other Professional Personnel:

E. Majchrowicz	Biochemist	LPS, NIAAA
E. Tamborska	Fogarty Visiting Fellow	BPB, NIMH
P. Morgan	Fogarty Visiting Fellow	BPB, NIMH
J. Deckert	Guest Researcher	BPB, NIMH
T. Insel	Staff Fellow	LCS, NIMH

I. Project Description

A. Objectives

Our understanding of the mechanisms involved in neural signaling has become much more sophisticated during the past five years. It is now obvious that neurons are able to use more than one chemical messenger to communicate with other neurons and that they are also receptive to multiple chemical messengers. Our conceptions of neurotransmitters have also changed, with it now being clear that multiple neuromodulators can affect the receptivity of a postsynaptic neuron to its neurotransmitter. The relatively recent characterization of several of the neuropeptides has revealed that these neuromodulatory agents may, in fact, be more important in regulating the activity of neural systems than the actual neurotransmitters themselves. Therefore, it might be more feasible to look for alterations in various neuromodulatory systems as the possible etiologic factors in psychiatric and neurologic disorders. This is especially true in light of the rather negative clinical findings that have been encountered in studies concerning neurotransmitter levels in various psychiatric disorders.

During the past five years rather substantial evidence has accumulated indicating that the purine nucleoside adenosine represents a unique example of a nonpeptide neuromodulator. Studies in our laboratory over this period of time have focused on the identification and characterization of adenosine receptors and uptake sites in brain. Our goal is to learn more about the molecular physiology of the adenosine system in the brain and how it modulates neural function. We are also interested in the potential for new drug development based on agents that will act specifically on the adenosine system; i.e., either the adenosine receptor or the adenosine uptake site. In this regard, our interests center on the anxiolytic and sedative properties of adenosine and adenosine derivatives, and how this system might interact with the benzodiazepine system. An additional interest of ours relates to developing specific ligand probes for the adenosine uptake site and to functionally characterize it. The relationship between the adenosine system, the "peripheral-type" benzodiazepine system, and calcium channels is also under study in our laboratory. A further objective of our studies is to determine the effect of other psychoactive drugs such as caffeine and carbamazepine on the brain adenosine system.

B. Methods Employed

Radioreceptor binding assays, surgery, drug treatment of animals, scintillation counting, labeling of ligands and autoradiography.

C. Results

During the past year, one of our major concerns has been the characterization of the adenosine uptake site in brain. The process of adenosine uptake is important since it is thought to be the major mechanism whereby the synaptic action of adenosine is terminated. Therefore, the properties of this site are of interest since its viability will in effect control the synaptic concentrations of adenosine and, consequently, the degree of adenosine receptor occupation. In our studies we have used [^{3}H]nitrobenzylthioinosine (NBI) as a binding probe for the adenosine uptake site protein. NBI had been shown previously to be a good inhibitor of adenosine uptake in erythrocytes and we decided some four years ago to determine whether this agent could be used as a probe for adenosine uptake sites in brain. Our group was the first to demonstrate specific, saturable and reversible binding of [^{3}H]NBI to brain membranes and to pharmacologically characterize

the site. During the past several years our work has been replicated by two other laboratories and both we and these other workers have generated substantial evidence that [^{3}H]NBI is binding to a site distinct from the adenosine receptor and that this site is probably, in fact, one of the functional purine nucleoside transport sites in the brain.

During the past year we have solubilized the adenosine uptake site and the adenosine receptor from both rat and guinea pig brain. We have shown that the two sites remain distinct pharmacologically in the soluble state, which indicates that they are, in fact, two separate entities and not simply the same site that is differentially associated with the membrane. Specifically, the adenosine uptake blockers such as dipyridamole, dilazep and hexobendine are several orders of magnitude more potent as inhibitors of [^{3}H]NBI binding to the solubilized preparation when compared to the adenosine receptor ligands such as cyclohexyladenosine (CHA) and diphenylxanthine (DPX). Also, caffeine does not inhibit [^{3}H]NBI binding to solubilized preparations whereas it does inhibit binding of [^{3}H]CHA to these same preparations. We have also shown that the kinetic properties of [^{3}H]NBI binding to the solubilized preparation is identical to that of the membrane bound site. We have, therefore, succeeded in preparing functionally intact adenosine uptake sites in the solubilized state. These studies are currently in press in the J. Neurochem.

One of our goals in solubilizing both the adenosine uptake site and the adenosine receptor was to purify each site and raise antibodies to them. Efforts directed at purifying the adenosine receptor have thus far been unsuccessful. We have tried, in collaboration with Drs. John Daly and Ken Jacobsen, to generate affinity columns for the receptor. The several columns that we have tested were not able to bind the solubilized adenosine receptor. We hope eventually to be able to separate the adenosine receptor from the uptake site. An ability to purify receptors is the next logical step in psychopharmacology research, but may realistically have to be delayed until we are able to hire a physical biochemist to work with our group.

During the past year, we have also completed a study of the adenosine uptake site in the heart. In this study, we compared the heart site with the brain site in dog. We are interested in the cardiovascular system because adenosine is a potent vasodilator and adenosine uptake inhibitors such as dipyridamole and dilazep are widely used clinically as antihypertensives. We showed in this study that the heart adenosine uptake site is similar to that in the brain and, more importantly, that the dihydropyridine calcium channel blockers such as nifedipine, nimodipine and nisoldipine are fairly potent inhibitors of [^{3}H]NBI binding in both the heart and brain. This data compliments studies we did several years ago when we showed that the calcium antagonists also inhibited binding of both agonists ([^{3}H]CHA) and antagonists ([^{3}H]DPX) to the adenosine receptor. It is, therefore, likely that the voltage-dependent calcium channel and the adenosine receptor and uptake site are associated with each other. One of our hypotheses is that adenosine exerts its effects via the inhibition of the intracellular mobilization of calcium. These data support that theory and have been published in Life Sci..

We have also completed an interspecies study of the adenosine uptake site. Here, we analyzed the pharmacology of the site in rat, mouse, dog, guinea pig and human brain. The findings showed that the pharmacology of the adenosine uptake

site is quite unique in the rat and mouse in that dipryidamole and hexobendine are much weaker inhibitors of [^{3}H]NBI binding in these species than they are in guinea pig, dog and human brain. It is, therefore, not optimal to use rat or mouse brain when dipyridamole is employed. The kinetics of [^{3}H]NBI binding across the five species tested was very similar. Calcium antagonists also were of similar potency as inhibitors of NBI binding to the adenosine uptake site, indicating that the coupling of adenosine uptake sites to the calcium channel is a general property of these sites. The interspecies study of adenosine uptake sites in brain has been published in Life Sci..

Efforts have also continued concerning the autoradiographic localization of adenosine uptake sites in brain. In these studies, we have used [^{3}H]NBI as the probe. In an effort to directly compare the localization of the uptake and receptor site, we also repeated the autoradiographic distribution of [^{3}H]CHA binding sites. The experiments were done by alternatively exposing serial sections to either [^{3}H]NBI (uptake site) or [^{3}H]CHA (receptor site). These studies showed a rather distinct distribution of the two sites but several areas such as the caudate, superior colliculus and the thalamus had high levels of both sites. Surprisingly, the molecular cell layer of the cerebellum and the hippocampus, two areas richly endowed with adenosine receptors, have very low levels of the uptake site. Brain areas that contain both the adenosine receptor and uptake site are currently the most likely candidates for having adenosinergic neurons. It is, however, important to apply another ligand for the uptake site since it is questionable whether NBI is labeling all of these sites. In this regard we are currently negotiating the preparation of [^{3}H] dipyridamole. Access to this reagent should greatly expand our ability to characterize the adenosine uptake system both at the anatomical and biochemical levels. Our autoradiographic studies of both the adenosine receptor and the uptake site have recently been published in the J. Neurosci.

We have continued our studies concerning the association of carbamazepine and the adenosine receptor. Previously, we showed that carbamazepine is a competitive inhibitor of both agonist and antagonist binding to the adenosine receptor. This past year these studies have been extended to show that chronic administration of carbamazepine (in the diet) causes a rather persistent upregulation of adenosine receptors in most brain areas. The increase in receptor number is still apparent three weeks after withdrawal of carbamazepine and suggests that this drug is acting as an adenosine antagonist. These studies have been submitted for publication in Epilepsia.

In related studies, we also managed to obtain from CIBA-Geigy some high specific activity tritiated carbamazepine. Attempts to directly demonstrate the binding of [^{3}H]carbamazepine to brain synaptosomal membranes have thus far proven to be unsuccessful. A low signal has been obtained but its lack of reproducibility, low affinity and our failure to convincingly show saturability have precluded definitive statements about the existence of a specific carbamazepine binding site in brain.

During the past year we have continued our long-standing interest in the benzodiazepine system. Recently, our studies have focused on the "peripheral-type" benzodiazepine binding site as labeled by [^{3}H]Ro5-4864. We have continued our study of this site in ethanol-treated animals and find an upregulation in the num-

ber of peripheral type sites in the cerebellum, hippocampus and cerebral cortex. This upregulation persists for seven days after cessation of alcohol treatment and, therefore, may be associated with some of the behavioral changes occurring during alcohol withdrawal. Contrary to what was stated by last year's report, we have not seen any changes in central type benzodiazepine receptors in these same animals. Multiple repeats of the protocol failed to reproduce the decreases in the central type receptor that we initially observed. These studies have been submitted to Life Sci. for publication.

Consistent with our long-term interest in the biochemical substrates of anxiety, we have studied several receptor systems in the brains of the Maudsley reactive strains of rats. We have focused on the benzodiazepine and adenosine system in these animals with the following findings. First, neither the central nor the "peripheral type" benzodiazepine receptors have shown any change in the reactive animals in four different brain areas. This directly contradicts a widely quoted preliminary report that appeared in 1979, which showed decreased benzodiazepine receptors. In an effort to set the record straight, we are preparing a paper for the J. Neurochem. to report our negative results.

Concerning adenosine receptors in Maudsley rats, we have found highly significant increases in the cerebellum of the reactive strain while no significant changes were observed in the cerebral cortex. An actual increase in the number of receptors was observed in the reactive animals. The rather selective upregulation of cerebellar adenosine receptors in the reactive animals has spawned a series of behavioral experiments which will investigate the sedative potency of various adenosine receptor ligands in the Maudsley reactive animals.

D. Significance to Biomedical Research and the Program of the Institute

The functional activity of brain neurons seems to be in large part controlled by the tone of several neuronal depressants including GABA, various peptides and adenosine. Further characterization of the adenosine system is, therefore, expected to provide insights regarding brain function at a very basic level and may well lead to the development of new psychoactive agents of clinical utility.

E. Proposed Course of the Project

Studies relating to adenosine receptors and uptake sites, the benzodiazepine receptor and calcium channels are expected to continue for several years.

Publications

Boulenger, J.-P., Marangos, P.J., Patel, J., Uhde, T.W. and Post, R.M.: Central adenosine receptors: possible involvement in the chronic effects of caffeine. Psychopharm. Bull. 20: 431-435, 1984.

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Verma, A. and Marangos, P.J.: Nitrobenzylthioinosine binding sites in brain: an interspecies study. Life Sci. 36: 283-290, 1985.

Bisserbe, J.C., Patel, J. and Marangos, P.J.: Autoradiographic localization of adenosine uptake sites in rat brain using [^{3}H] nitrobenzylthioinosine. J. Neurosci. 5: 544-550, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00450-11 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological Rhythms in Affective Illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	W. C. Duncan	Research Psychologist	CPB/NIMH
	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Biological rhythm disturbances have been described in patients with depression. Experimental manipulation of these rhythms by sleep deprivation, or phototherapy can produce marked changes in mood, suggesting that alterations in the circadian rhythms of patients with affective disorders play an important role in the pathophysiology of these disorders. As we have noted in our previous annual report Z01 MH 00450 10 CP, circadian oscillators cannot be measured directly. In our present study a more precise description of the intrinsic properties of the biological clocks in depression are obtained by observing patients and normal controls under constant conditions of diet, physical activity, wakefulness, and illumination.

Thus far we have studied 12 patients and 7 age and sex-matched normal controls. Preliminary data from our study of neurotransmitter metabolites indicates that a previously described rhythm in the norepinephrine metabolite MHPG in the plasma is the result of external factors. Although no rhythm in MHPG was found, depressed patients had significantly lower plasma MHPG than controls and this difference could not be attributed to physical activity, diet or disrupted sleep. By contrast, a circadian variation in dopamine metabolite, HVA, was observed with peak values seen at night, which persists under constant conditions. No patient normal differences were noted in this variable.

Already this design appears useful in discriminating between circadian variability arising from intrinsic versus extrinsic factors and the study of physiologic variables under constant conditions has implications beyond circadian research.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

The present project and its methods were extensively described in Z01 MH 00450-10 CP.

Methods:

Patients are included if they meet RDC criteria for major affective disorder (UP, BPI or BPII), and they are free of all psychotropic medications for at least three weeks.

Prior to beginning this study, subjects are adapted to an indwelling venous catheter, rectal temperature probe, wrist activity monitor and psychological testing. On the baseline day specimens will be obtained every thirty minutes for cortisol and melatonin and every two hours for neurotransmitter metabolites via the indwelling catheter. The subject is ambulatory, in ordinary room lighting and on the regular ward diet. Psychological testing, mood ratings and a sleepiness rating are obtained hourly. Activity is measured by wrist activity monitor and core body temperature obtained by continuous monitoring of rectal temperature stored and recorded by computer every five minutes.

Beginning on the second day of the study room lighting is limited to 100 lux and an isolocaloric liquid diet in equal hourly feedings is substituted for regular diet. Subjects are kept at bedrest and are continuously awake from the morning of the second day through noon of day three in order to control for differences arising from sleep and also to assess the clinical response to sleep deprivation. All previous measures are repeated on the second and third days.

Findings to date:

Thus far 12 patients and 7 normal controls have completed the present study. Data has been analyzed for the neurotransmitter metabolites HVA, and MHPG in plasma in seven bipolar depressed patients and seven age-match controls. Plasma HVA shows a circadian variation with peak values occurring at night and the lowest values occurring in the afternoon. Under constant conditions, HVA is significantly lower in both patients and normal controls but this decrease predominantly occurs in the morning whereas nighttime peaks are unaffected. No difference in plasma HVA was observed between patients and controls.

At baseline, plasma MHPG showed a significant circadian variation with values increasing in the afternoon and peaking before bedtime. Under constant conditions no circadian variation in MHPG was present. Mean MHPG at baseline did not differ between patients and controls or under constant conditions.

Our findings suggest that: 1) There is a circadian rhythm in plasma HVA but that the apparent rhythm in MHPG is the result of masking. 2) We failed to find any difference in the timing (or phase) of the HVA rhythm in patients compared with controls.

2. It will provide additional evidence for extending or revising the present theoretical formulations regarding circadian rhythms in affective and sleep disorders.

3. It will establish whether a relationship exists between abnormalities described in neurotransmitters and their metabolites as measured in single time point studies of depressed patients and the circadian rhythms for these neurotransmitters.

4. It will determine whether the antidepressant effect to sleep deprivation is related to the circadian abnormalities seen in these patients.

Proposed Course:

A few additional patients will be sought for long-term, longitudinal studies. Much data, especially concerning the possible interrelationship between sleep EEG and temperature, remains to be analyzed.

Publications:

Wehr, T.A.: Biological rhythms and manic-depressive illness, in Ballenger, J.C., Post, R.M. (eds.), Neurobiology of the Mood Disorders, Williams and Wilkins, Baltimore/London, 1984.

Sack, D.A., Rosenthal, N.E., Ashburn, E., Wehr, T.A.: The potentiation of antidepressant medications by phase-advance of the sleep-wake cycle. American Journal of Psychiatry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02192-03 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sleep in Psychiatric and Endocrine Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	D. Sack	Chief, Inpatient Services	CPB/NIMH
	B. Parry	Clinical Associate	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

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Unit on Sleep Studies

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.3	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has become a part of other Branch projects, including Z01 MH 02203-02 CP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02193-03 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies of Insomnia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.3	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our work has suggested that insomniacs may have cognitive disorders by day, manifested by a decrease in semantic memory (the ability to retrieve or use material already well learned) and also suggested that cognitive processes during sleep may also be altered. The current study has pursued this possibility, by testing arousal thresholds to meaningless and meaningful stimuli during sleep, ability to respond to commands during sleep, and similar measures. Among our tentative findings are the observation that insomniacs have decreased ability to differentiate between meaningless and meaningful stimuli during sleep.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

10 insomniacs and controls underwent a series of studies including a polysomnogram to rule out physiologic disorders of sleep, a multiple sleep latency test, and a series of cognitive tests during sleep.

Methods:

Among the testing procedures were:

1. response to meaningless stimuli. At 5 polysomnographically-defined points during sleep, a tone of progressively increasing volume was played in order to determine the volume resulting in EEG wakefulness.

2. response to meaningful stimuli. At 5 points during sleep, a tape recording of a voice calling out the subject's name was played at progressively louder levels.

3. classical conditioning. During sleep a conditioning procedure was performed, in order to assess the subject's ability to take in and act on information provided during sleep.

4. response to commands from wakefulness. During wakefulness subjects were told that during sleep they should push a button whenever they hear a tone.

Findings to Date:

Our data so far suggest that at sleep onset (10 minutes after the first spindle), the arousal threshold for meaningful stimuli remains relatively constant for normals and insomniacs. For meaningless stimuli, however, the normals are able to achieve rising thresholds, while insomniacs are not. We have also found tentative evidence that during REM sleep the insomniacs are able to respond to the button-pushing tasks more effectively than normals. This would seem to fit with our previous finding that insomniacs consider REM sleep to be a 'lighter' stage than do normals.

Significance to Biomedical Research and to the Program of the Institute:

These data continue to suggest that insomnia, which is a chronic problem to perhaps 10% of the population, may be related to altered cognitive processes both when awake and asleep.

Proposed Course:

We continue to pursue the possibility that more effective non-pharmacologic approaches may be useful to treat insomnia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02200-03 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Light Suppression of Nocturnal Human Melatonin Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
Others:	S. P. James	Clinical Associate	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH
	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Melatonin (MT) is secreted by the pineal gland (PG) almost exclusively at night. Our previous work has shown that MT is present in humans and that its nocturnal secretion can be suppressed with bright artificial light (>2000 lux). This effect of light is presumed to be mediated by neural pathways connecting the retina to the PG via the hypothalamus. At the hypothalamic level the effect of light is thought to be mediated by nicotinic cholinergic receptors. Suppression of MT is presently the only index of hypothalamic sensitivity to light. We have shown that humans have a high threshold for light-MT effects compared with experimental animals. Ordinary artificial light, for example, appears ineffective in humans.

Abnormal hypothalamic sensitivity to light may be an important trait and pathogenic mechanism in manic-depressive illness (Project Z01 MH 02199-01 CP), seasonal affective disorder (Projects Z01 MH 02205-01 CP and Z01 MH 02206-01 CP) and delayed sleep phase syndrome (Projects Z01 MH 02196-01 CP and Z01 MH 02197-01 CP).

The purpose of this project is (1) to standardize the light-MT suppression test (LMST), (2) to identify sources of variance in the LMST (age, sex, prior sleep or waking, prior exposure to light, etc.), and (3) to investigate hypothalamic sensitivity to light using the LMST in the disorders outlined above.

We have standardized the administration of light by using high-intensity tungsten lamps. Spectral qualities are controlled by ultraviolet and infrared filters modifying the light projected into a ganzfeld dome, and intensities of light are varied by using neutral density filters. Blood samples are obtained before, during and after light administration. Using these methods a fluence-response curve for the LMST is being generated with the degree of MT suppression expressed as a function of log light intensity (watts/cm^2).

Project Description:

Melatonin (MT) is formed from serotonin by N acetyl transferase (NAT) and 5-hydroxy-O-methyl transferase (5HOMT) in the pineal gland (PG) and is released into the blood and CSF. Melatonin secretion is stimulated by neural impulses originating in the suprachiasmatic nucleus (SCN) of the hypothalamus and transmitted through sympathetic outflow to the superior cervical ganglion (SCG) and thence to the PG. In all species MT is secreted almost exclusively at night. Its cyclic nocturnal secretion is driven by the clocklike behavior of the SCN, a circadian rhythm pacemaker. Nocturnal secretion of MT can be suppressed by exposure to light. Light acts on the SCN via the retino-hypothalamic tract (RHT). Intraventricular carbachol, a cholinergic nicotinic agonist mimics the effects of light on the SCN; therefore the RHT-SCN innervation is thought to be cholinergic. Cholinergic synapses also occur in the SCG. The SCG innervates the PG via noradrenergic fibres and post-synaptic beta receptors. MT secretion is stimulated by beta agonists and inhibited by beta blockers.

Our previous work, using a gas chromatograph mass spectrometic (GCMS) assay, has shown that (1) MT is present in human blood, (2) is secreted mostly at night, (3) is suppressed by beta blockers, and (4) is suppressed by bright artificial light. We showed that the MT rhythm is synchronized poorly or not at all with the day-night cycle in blind persons. We also found that manic-depressive patients appear to be supersensitive to the MT suppressing effects of light, regardless of clinical state.

The hypothalamus-pineal axis was previously believed to have a very high threshold for suppression by light in humans compared with animals (2000 lux versus 10-50 lux). Preliminary results from this study suggest that humans are more light sensitive than earlier suspected, although the human threshold is such that artificial light ordinarily present in home and work environments has little effect.

There is theoretical and empirical support for the hypothesis that abnormal hypothalamic sensitivity to light is a trait and a pathogenic mechanism in manic-depressive illness (Project Z01 MH 02199-01 CP), seasonal affective disorder (Projects Z01 MH 02205-01 CP and Z01 MH 02206-01 CP) and delayed sleep phase syndrome (Projects Z01 MH 02196-01 CP and 02197-01 CP).

The purpose of this project is (1) to standardize the light MT suppression test (LMST), (2) to identify sources of variance in the LMST, and (3) to use the LMST to investigate hypothalamic sensitivity to light in the disorders outlined above.

Methods:

Light is projected from a tungsten bulb into a ganzfeld dome which is a sphere that allows the subject to be exposed to a homogenous field of illumination. The intensity of light is controlled by the use of neutral density filters, and the actual amount of light projected into the sphere is measured by a lux photometer. Blood samples are obtained before, during, and after light exposure.

Proposed Course:

Light will be administered in a quantitative manner using a tungsten lamp with infrared and ultraviolet filters. The light will be projected through neutral density filters into a ganzfeld dome, which is a sphere that enables the subject to gaze into a homogenous field of illumination. Patients will look into the dome from 2 a.m. to 3 a.m. and be exposed to indirect light. Blood samples will be obtained from subjects before, during, and after exposure to light.

Using this method a fluence response curve for the LSMT will be obtained in which degree of suppression is expressed as a function of log light intensity (watts/cm^2).

The following possible sources of variance will be investigated: height, weight, age, sex, phase of menstrual cycle, prior sleeping and waking, and prior exposure to light.

Subsequently the various diagnostic groups will be investigated.

Findings to date:

Seven healthy males were exposed to light in the ganzfeld dome from midnight to 1 a.m. once a week for four weeks. Each week a different intensity of light (200 lux, 1000 lux, 1500 lux, 2500 lux) was used in a randomized process. Suppression of melatonin after one hour of exposure was identical with 1000 lux, 1500 lux and 2500 lux while the 200 lux did not show suppression. These findings suggest that humans are more light sensitive than previously believed.

Significance to Biomedical Research and to the Program of the Institute:

1. Preliminary findings with the LMST indicate abnormal hypothalamic sensitivity to light in manic-depressive illness. To have confidence in these results, the studies must be repeated using a more sensitive and quantitative method of administering light.

2. Abnormal hypothalamic sensitivity to light, if present in delayed sleep phase syndrome, manic-depressive illness or seasonal affective disorder, could have pathogenic significance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02201-03 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Early Versus Late Partial Sleep Deprivation in the Treatment of Depression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
Others:	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH
	W. B. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In our previous study of partial sleep deprivation (PSD), described in detail in Z01 MH 02201-02 CP, we observed that partial sleep deprivation was more effective if subjects were kept awake in the latter part of the night, i.e. after 2 a.m., than if they were kept awake earlier in the night. Patients also slept less on this condition, and the amount of sleep was inversely related to improvement. Thus from our earlier study it is not possible to know whether the timing of sleep is an essential factor in the antidepressant effects of PSD, but it appears that the amount of sleep reduction, and in particular the reduction in REM sleep, are correlated with clinical response. We also observed in our previous study that the antidepressant effects of a single night of PSD could be enhanced and extended by a second night of treatment and we have hypothesized that repeating PSD over an extended period might produce antidepressant effects comparable to other somatic therapies.

Our present work with partial sleep deprivation consists of two studies. The first is a replication of our earlier project in which we compare the efficacy of sleep deprivation early in the night, i.e. before 3 a.m., with PSD late in the night after 3 a.m. In the present study the duration of sleep on the two treatments is precisely regulated so that unequal sleep durations will not be a source of variance on the different conditions. The second study is a clinical trial of repeated partial sleep deprivation treatments, performed over a three week period. This preliminary study will enable us to assess whether partial sleep deprivation may be useful in the clinical management of depressed patients.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

The timing of various circadian rhythms appears to be shifted to an abnormally early time in depression, raising the possibility that the timing of sleep relative to circadian rhythms (their internal phase relationship) is a pathogenic factor in affective illness. The antidepressant effects of partial sleep deprivation may, therefore, depend on the time at which sleep occurs. The objectives of this study are:

1) To determine the efficacy of partial sleep deprivation in the first half versus the second half of the night when the duration of sleep is precisely controlled.

2) To describe the diagnostic, biochemical neuroendocrine and psychophysiologic predictors of the sleep deprivation response.

3) To determine the effects of sleep deprivation on neurotransmitters whose function is thought to mediate other antidepressant responses.

Methods:

Patients must meet RDC criteria for a major affective disorder, either unipolar or bipolar, and must be free of all psychotropic medications for at least three weeks. Prior to treatment with PSD patients undergo baseline evaluations which include the following: 1) EEG recording of sleep, 2) 24 hour rectal temperature monitoring, 3) examination of neurotransmitters and their metabolites in plasma, urine, and spinal fluid, 4) circadian study of plasma hormones. All subjects will participate in a random order, crossover study comparing the acute effects of PSD-L and PSD-E similar in design to that described in Z01 MH 02201-02 CP. Following this study patients will be treated with PSD for three weeks, with two treatment nights alternating with one night of recovery sleep during that period.

Significance to Biomedical Research and to the Program of the Institute:

1. If the results of earlier experiment are confirmed and PSD is effective only when wakefulness occurs after 3 a.m. this would suggest that a sleep-dependent process occurring in the second half of the night plays an important role in the pathophysiology of depression. Alternatively it could also indicate that interruption of sleep at that hour initiates neuronal changes which counteract depressed mood. An understanding of these processes could lead to other new treatments for depression.

2. We have hypothesized that extended treatment with PSD will have sustained antidepressant effects enabling this treatment to be adapted to a variety of clinical settings. In addition to providing a new treatment for patients who are not responsive to or unable to tolerate antidepressant medications this treatment would be a valuable research tool investigating changes in mood without the confounding effects of medications.

Proposed Course:

This year we expect that our experimental design will enable us to more effectively control for sleep duration as variable in the PSD response.

We also hope to determine whether repeating PSD over an extended period of time has sustained antidepressant effects similar to these with tricyclic antidepressants and MAO inhibitors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02202-03 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Features of Seasonal Affective Disorder (SAD)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
Others:	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
	F. M. Jacobsen	Clinical Associate	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH
	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously described the syndrome of seasonal affective disorder (SAD), a condition characterized by recurrent winter depressions alternating with remissions or hypomania in the spring and summer. During the past year we have extended our observations particularly in the recognition and description of children and adolescents with this condition and the specific way in which they manifest the disorder. We have asked parents to rank in importance the symptoms they have noted during the fall and winter months and are in the process of devising a rating scale to measure changes in these specific symptoms. During the coming year we are planning to collaborate with Dr. William Sonis in the Department of Psychiatry at the University of Minnesota to further characterize this disorder in children. We are also continuing to collect clinical and demographic information about all new adult patients admitted to our program. However, the consistency in the descriptive information on patients over the past 3 years suggests that new data are likely simply to corroborate those data already collected.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

The clinical features of SAD in adults have been well described. We have encountered 7 children and adolescents with symptoms of SAD and have set out to characterize the nature of these symptoms.

Methods:

Subjects came to our attention either as a direct response to newspaper articles or were referred by a parent who was participating in our program. In order to be included patients had to be less than 18 years old and have a history of at least two consecutive winters during which they experienced sustained periods of decreased energy, school difficulties and evidence of mood problems. We asked parents to rank order the commonest symptoms according to severity along a "most troublesome - least troublesome" dimension. We then ranked the symptoms in order of severity for the population as a whole.

Findings to date:

Of the 7 children, 4 were boys and 3 were girls. Five were children of adult participants in our program. Their ages ranged from 6 to 17 years and the ages of onset of symptoms ranged from 2 to 10 years. Symptoms ranked in order of prominence were: irritability, fatigue, school difficulties, sadness, sleep changes (usually increased sleep length), headaches, appetite changes, decreased activity, carbohydrate craving, crying spells, anxiety, social withdrawal, and temper tantrums.

Significance to Biomedical Research and to the Program of the Institute:

The recognition of mood and behavioral difficulties in children and adolescents as falling into the spectrum of SAD has important practical implications. Teachers and counsellors should be aware of the problem as one of the differential diagnoses of school difficulties in the fall-winter semester. Such recognition is particularly important since this syndrome can be easily reversed by exposing the affected individuals to enhanced environmental lighting (see annual report Δ Z01 MH 02205-02 CP). It is to the credit of the program that the first report on this syndrome originates from Intramural Research Program.

Proposed Course:

We will be collaborating with Dr. William Sonis of the Department of Psychiatry of the University of Minnesota, who is in the process of recruiting a population of children and adolescents with SAD at present.

Publications:

1. Rosenthal, N.E. Seasonal rhythms in mood and behavior, In: Symposium: Endocrine Rhythms and Behavior. Annals of the Royal College of Physicians

and Surgeons of Canada, 17 (7): 599-602, 1984.

2. Rosenthal, N.E. Seasonal incidence of depression. Human Sexuality, 19 (4):125, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02203-03 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sleep, Temperature and Activity Changes in Women with Premenstrual Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
Others:	B. L. Parry	Clinical Associate	CPB/NIMH
	W. B. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	N. E. Rosenthal	Chief, Outpatient Studies	CPB/NIMH
	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The symptoms of premenstrual syndrome (PMS) consist of mood, cognitive, and behavioral disturbances occurring in the premenstrual phase of the menstrual cycle. They may become severe enough to cause suicidal depression or psychosis. Objective physiologic parameters that correlate with the subjective symptoms of PMS need to be identified in order to delineate this syndrome further and possibly to suggest better forms of treatment. First, this study examined sleep, temperature and activity changes across the menstrual cycle in women with moderate to severe premenstrual syndrome and in normal volunteers. Two months of baseline activity recording, objective ratings and self-ratings of sleep, mood, and energy were obtained. Subjects were then admitted to the hospital where they underwent sleep EEG and temperature recordings two nights a week for the duration of one menstrual cycle.

Premenstrual syndrome may represent a variant of affective disorder. Therefore, treatment modalities found to be effective in the major affective disorders may be useful in treating patients with PMS. For example, sleep deprivation which induces transient remissions in affective disorder may do the same in PMS. Furthermore, sleep deprivation lowers prolactin, and hyperprolactinemia has been associated with mood disturbances in patients with PMS. Therefore, the effects of sleep deprivation were investigated in these patients. Prolonged intense light exposure alleviates symptoms in patients with seasonal affective disorder. Since symptoms of SAD and PMS are similar, prolonged intense light exposure or treatment with the beta blocking drug, atenolol, which, like bright light, suppresses melatonin was evaluated as a possible treatment for PMS.

Results of sleep deprivation and light treatment experiments may increase our understanding of the pathophysiological mechanisms of PMS and the relationship between PMS and affective disorders.

Project Description:

See Annual Report Z01 MH 02203-02 CP, 1984.

Methods:

See Annual Report Z01 MH 02203-02 CP, 1984.

Findings to date:

Forty individuals were screened for the following protocols:

A) Sleep studies across the menstrual cycle: 8 women with PMS and 8 age matched normal volunteers underwent sleep EEG studies two nights a week for the duration of the menstrual cycle. The sleep data is currently being analyzed, but preliminary findings indicate changes in total sleep time, delta sleep, and REM sleep across the menstrual cycle and differences between patients and controls.

B) Twelve patients with PMS underwent total sleep deprivation (TSD) during the premenstrual phase of their cycle, 8 of whom had a therapeutic response. Furthermore, four of the patients who responded to total sleep deprivation underwent partial sleep deprivation in the first (PSD-E) or second half (PSD-L) of the night. All patients undergoing (PSD-L) had a therapeutic response similar to that of TSD.

C) Light studies: One patient with seasonal PMS, i.e., who had premenstrual symptoms only in the winter, had a therapeutic response to high intensity light treatment (evening hours only), which was blocked by simultaneous administration of melatonin, and also achieved by administration of propranolol and atenolol, drugs which block the synthesis and release of melatonin. Based on this finding, a double blind randomized, crossover trial of atenolol was undertaken in non-seasonal PMS patients. The study is currently in progress and 8 patients have completed the study. Preliminary analysis of the data does not suggest a marked effect of atenolol on PMS patients.

Significance to Biomedical Research and to the Program of the Institute:

PMS is responsible for morbidity in 40-60% of the female population. No objective physiologic manifestations of PMS have been identified that could be used to follow its course and to measure its response to treatment. Furthermore, current pharmacologic treatment modalities have not been consistently effective and are fraught with side effects. Changes in sleep, temperature and activity across the menstrual cycle may prove to be useful physiological markers of PMS symptoms; also, sleep deprivation or light therapy may be an effective non-pharmacologic treatment. On a conceptual level, the study may elucidate the role of changes in sleep, temperature, activity, and biological clocks in the pathophysiology of PMS.

Proposed Course:

Should total sleep deprivation be an effective clinical treatment of PMS, more specific alterations of sleep schedules such as partial sleep deprivation (early vs. late), or shifting the time of sleep will be applied. Should the clinical effects of sleep deprivation be correlated with the lowering of serum prolactin, then a series of pharmacological studies will be done to determine whether or not prolactin inhibition mediates the sleep deprivation response. For example, if nighttime infusions of L-DOPA, which lowers prolactin, are given when subjects are asleep, will this also produce the same antidepressant response as sleep deprivation? Also, if TRH is given acutely, or neuroleptics chronically, to increase prolactin while patients are sleep deprived, will this counteract the antidepressant response of sleep deprivation? Should "light therapy" be effective in ameliorating premenstrual symptoms, a study of women's sensitivity to light across the menstrual cycle will be undertaken to determine whether it might be a state or trait marker.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02205-03 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Light Interventions in Seasonal Affective Disorder (SAD)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
Others:	F. M. Jacobsen	Clinical Associate	CPB/NIMH
	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH
	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS *(if any)*

Fairbanks Neurological and Psychiatric Clinic, Alaska, C. Hellekson
Department of Biochemistry, University of Surrey, England, J. Arendt
Department of Psychiatry, University of Minnesota, B. Sonis

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.4	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously shown in four separate groups of adult patients with seasonal affective disorder (SAD) that exposing these patients to bright full-spectrum light for 3 hours in the morning and 3 hours in the evening, or for 5 hours in the evening alone, produces marked antidepressant effects. In this past year we examined whether the antidepressant effects are mediated via the photoperiod, i.e. whether they depend on extending the normal daylength or whether they will occur even if the light exposure takes place within normal daylight hours. We treated 7 inpatients with SAD in a crossover design involving two 1-week light manipulations separated by a week off lights. The 2 lighting manipulations both involved 2 3-hour periods of bright (2500 lux) light, separated either by 2 hours of dim light (short skeleton photoperiod) or 9 hours of dim light (long skeleton photoperiod). Based on animal studies we predicted that the latter would have antidepressant effects in our patients but that the former would not. In fact, we found the treatment schedules to be equally efficacious, which would argue against a photoperiodic mechanism for the antidepressant effects of bright environmental light in SAD.

We collaborated with Dr. Carla Hellekson in Fairbanks, Alaska, who showed in 6 patients with SAD that two hours of bright light in the morning, two hours of bright light in the evening, and two hours of bright light divided equally between morning and evening were all equally effective in treating patients with SAD.

In an uncontrolled study we showed that increasing the environmental light had significant antidepressant effects in 6 children and adolescents with SAD. In further studies we are continuing to explore the formal properties of light treatment in SAD.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description:

Previous studies have shown that extending the photoperiod with bright full-spectrum light has antidepressant effects in patients with seasonal affective disorder (SAD). During the past year we investigated the effects of giving pulses of bright light at different times of the day to determine whether the antidepressant effects of light are photoperiodic (i.e. dependent on the timing of light exposure) or whether the effects will occur regardless of when the light exposure takes place.

In a collaborative study with Dr. Carla Hellekson in Alaska we again studied whether timing of light treatments is important. We also studied, in an uncontrolled way, the effects of light modification in 6 children and adolescents with SAD.

Methods:

1. The Skeleton Photoperiod Study: Patients were recruited via the media and community referrals. They were screened for a history of SAD and were followed clinically into the winter months. When they became depressed, they were admitted to our inpatient unit and entered the first week of a crossover study. Thereafter they were discharged and remained off light treatment for a minimum of 10 days, at which time they were admitted for the second week of treatment provided they were depressed at that time. In some cases, where the first treatment had been particularly effective, a longer wait was necessary before relapse occurred. The two treatments, given in random order, consisted of two 3-hour periods of bright (2500 lux) environmental light separated by either 3 hours of dim light (short skeleton photoperiod) or 9 hours of dim light (long skeleton photoperiod). Mood was measured by means of the Hamilton Rating Scale (HRS) by interviewers blind to the type of treatment administered after each week of treatment and after each withdrawal period. The patients were kept in a room in controlled dim lighting at all times except when the bright light was being administered or when they were asleep. Duration and timing of sleep was held constant across the two conditions. Twenty-four hour urine specimens were collected under both conditions and analyzed for the melatonin metabolite, 6-hydroxymelatonin sulphate (6-OH-MT-S).

Based on the animal literature involving seasonal rhythms, which can be modified by light, which have been found to be photoperiodic, and which have been found to be mediated via the hormone, melatonin, we predicted that the long skeleton photoperiod would be effective in reversing the winter symptoms of SAD whereas the short skeleton photoperiod would not. We also predicted that the long photoperiod would suppress the secretion of melatonin, and therefore its metabolite, 6-OH-MT-S, to a greater degree than the short photoperiod.

2. The Alaska Study: Dr. Hellekson recruited patients via the news media and screened them by means of a questionnaire and clinical interview. In the winter months, when patients became depressed, as determined by

administration of a videotaped Hamilton Rating Scale (HRS) by Dr. Hellekson, patients entered a crossover study during which they had 3 one-week light treatments separated by at least a week off lights. The 3 treatments consisted of 2 hours of bright (2500 lux) full-spectrum light administered either in the morning upon arising, in the evening, or divided equally between morning and evening. HRS scores were determined by Dr. Hellekson after each treatment and withdrawal period. Wherever possible the HRS interviews were videotaped and rated by "blind" clinicians from our group.

3. The Child Study: In an uncontrolled trial, 5 out of the 6 children were exposed to bright full-spectrum light for 1 to 3 hours per day.

Findings to date:

1. The Skeleton Photoperiod Study: Seven patients in all were studied. Contrary to our prediction, both long and short skeleton photoperiod treatments produced significant antidepressant effects. However, the urinary secretion of 6-OH-MT-S after the long photoperiod treatment was significantly less than that found after the short photoperiod treatment. This preliminary evidence suggests that the antidepressant effects of light are not photoperiodic and that they are probably not mediated via the effects of light on melatonin.

2. The Alaska Study: There was a high degree of reliability between blind and non-blind raters (intraclass coefficient = 0.9). All three types of treatment were equally effective. However, there was an ordering effect and the third treatment, which was administered as the days were getting longer, was significantly less effective than the first two.

3. The Child Study: Five out of six children and and adolescents who received light treatment showed some benefit, that was observed by parents, teachers and the children themselves. Our preliminary impression was that the children seemed to require less light exposure than the adults we have treated.

Significance to Biomedical Research and to the Program of the Institute:

1. If, indeed, the antidepressant effects of light in SAD are not photoperiodic, as is suggested by the results of the skeleton photoperiod study, this has important practical and theoretical implications. Since all seasonal behaviors in animals, which are mediated photoperiodically have been shown to involve the pineal gland, the results of our skeleton photoperiod study throw into question whether the antidepressant effects of light are mediated via melatonin and even whether the relevant neuroanatomical pathways may bypass the eye. It would be of clinical and theoretical value to know how these effects are mediated and such knowledge would suggest new research strategies. It is appropriate that these questions and strategies should arise within the Intramural Program where the syndrome of SAD and its treatment with light were first developed.

If it is not necessary to extend the photoperiod in order for light treatment to be effective, it may be possible to treat patients during the day while they are at work.

2. The Alaska study also offers both theoretical and practical insights. It is important for the clinician to know whether it matters when during the day the light is given. Lewy and colleagues have suggested that it is very important that light be administered in the morning hours. These data would argue against that point of view. It is useful for our program to have data which speak to this question.

3. The finding that children with mood and behavioral difficulties in the fall semester can have their problems reversed simply by exposing them to bright environmental light for a few hours during the day is clinically important. Light is easy to administer and is not toxic when adminstered correctly; yet the benefits may be considerable. This is the first time that seasonal problems and light treatment in children have been described.

Proposed Course:

We plan to continue to explore the mechanisms by which light treatment works. We are considering administering light to the skin and to the eye in different treatment conditions within the same subjects to evaluate the anatomical pathways involved. We are also considering a trial of light treatment during the daytime hours.

We plan to continue our collaboration with Dr. Hellekson in Alaska. As far as continued studies on children is concerned, we are planning to collaborate with Dr. William Sonis at the University of Minnesota. They are collecting a population of children with SAD and are planning to undertake a study of light treatment in these children.

Publications

1. Rosenthal, N.E., Sack, D.A., Carpenter, C.J., Parry, B.L., Mendelson, W.B., Wehr, T.A. Antidepressant effects of light in seasonal affective disorder. Am J of Psychiatry, 142 (2):163-170, 1985.

2. Rosenthal, N.E., Sack, D.A., James, S.P., Parry, B.L., Mendelson, W.B. Seasonal affective disorder and phototherapy. Proceedings of New York Academy of Sciences, Conference on Medical and Biological Effects of Light (In Press).

3. James, S.P., Wehr, T.A., Sack, D.A., Parry, B.L., Rosenthal, N.E. Evening light treatment of seasonal affective disorder. British Journal of Psychiatry (In Press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02206-03 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurobiology of Seasonal Affective Disorder (SAD)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
Others:	L. Tamarkin	Research Biologist	CPB/NIMH
	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH
	W. B. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
	F. M. Jacobsen	Clinical Associate	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.4	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As we have described elsewhere, patients with seasonal affective disorder (SAD) have annually occurring cycles of depression in fall and winter alternating with euthymia or hypomania occurring in the spring or summer. In the past we have investigated the neurobiology of patients with SAD by studying their sleep and neuroendocrine functioning. We have found that they sleep longer and have reduced delta sleep in the winter, as compared with the summer. However, dexamethasone suppression of cortisol secretion and TSH release in response to externally injected TRH appear normal.

This past year our neurobiological investigations have revolved around melatonin metabolism in SAD. Blood was drawn over a 48-hour period in 7 SAD patients and 7 normal volunteers both in summer and in winter in order to establish the pattern of melatonin secretion in patients and normals. In addition, the melatonin precursor, 5-hydroxy-tryptamine was administered orally to 10 patients and 10 normal volunteers in the winter months and blood was drawn over a 5 hour period for melatonin analysis. We hypothesized that perhaps SAD is characterized by melatonin overproduction and that such a load of melatonin precursor would result in greater production of melatonin in patients than in normals. The melatonin specimens have yet to be analyzed.

Project Description:

Since seasonal affective disorder (SAD) was first described, we have been curious about the neurochemical basis of the abnormal reaction to the changing seasons and about the mechanism of the effects of phototherapy. Melatonin, an substance secreted by the pineal gland, is of known importance in mediating seasonal rhythms in animals and the effects of light on these seasonal rhythms are generally mediated via their effects on melatonin. It is therefore of interest to look at the pattern of melatonin secretion in the plasma in patients with SAD both in a naturalistic setting and following a challenge to melatonin production. We chose as the challenge paradigm to administer the melatonin precursor, 5-hydroxytryptamine (5HTP), as this has been shown to enhance melatonin secretion in sheep during the day time when such secretion usually does not occur.

Seven SAD patients and 7 normal volunteers had blood drawn for 48 hours under dim light conditions both in summer and in winter. Ten SAD patients and 10 normal volunteers were given and oral dose of 5HTP and of placebo during the winter months and blood was drawn over a 5 hour period for melatonin measurement. Patients rated mood and drowsiness following these challenge studies.

Findings to date:

There was no effect of 5HTP on mood or drowsiness in either patients or normals. There are no other findings to date as the specimens await analysis for melatonin content.

Significance to Biomedical Research and to the Program of the Institute:

It would be of great value to establish the biological mechanisms which underlie abnormal responses to seasonal changes and the reversal of these responses by exposure to bright artificial light. This might enable us to find simpler and more convenient types of treatment and also to understand the physiology of our neurological responses to our environment, which might have significance in a variety of populations including non-seasonal depressives and patients with eating and sleep disorders.

It is not possible at this time to evaluate the studies in which melatonin measurement is the crucial dependent variable since our samples have not as yet been analyzed. Other work performed by our group this past year suggests that melatonin may play a less important role in SAD and phototherapy than we had previously thought, leaving open the question as to what other neurotransmitter or hormone systems may be important in this regard.

Proposed Course:

Clearly our proposed course with regard to the role of melatonin in SAD will have to await the results of the analysis of the samples already collected.

We are planning to study the possible role of other neurotransmitters and peptides. We are planning to conduct studies of cerebrospinal fluid in patients before and after light treatment and to measure a variety of substances which have been thought to be of importance in the affective disorders. We are specifically planning to study the possible role of serotonin in mediating the symptoms and effects of phototherapy. Several sources of information suggest that serotonin may be of importance in SAD. First, serotonin deficiency has long been postulated to be an underlying abnormality in depression in general. Second, Carlsson and colleagues have shown a seasonal variation in hypothalamic serotonin in the brains of people who have died from non-psychiatric, non-neurological disorders; serotonin levels drop significantly in the fall and winter months. Third, it has been suggested that the carbohydrate craving, a symptom which is characteristic of SAD, may be related to a deficiency of brain serotonin. This latter suggestion stems from work by Wurtman and colleagues, who have shown that serotonin synthesis is related to the ratio of tryptophan to other amino acids in the plasma, and that carbohydrate intake influences this ratio in such a way as to increase the passage of tryptophan into brain and thus increase serotonin synthesis. The carbohydrate craving might be part of a complex behavioral-biochemical feedback loop in which carbohydrate ingestion restores deficient levels of brain serotonin. We are planning to test this hypothesis by feeding SAD patients and normals various amino acid combinations calculated either to decrease or increase brain serotonin levels, and to evaluate the effects on mood and carbohydrate craving.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02221-02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Melatonin in Seasonal Affective Disorder (SAD)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
Others:	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
	F. M. Jacobsen	Clinical Associate	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH
	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
	W. B. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	L. Tamarkin	Research Biologist	CPB/NIMH

COOPERATING UNITS *(if any)*

Department of Biochemistry, University of Surrey, England, J. Arendt

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	0.8	1.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Last year we showed that exogenously administered melatonin reinduced some but not all the symptoms of SAD, which had responded to phototherapy in 8 patients with SAD. This year we treated 18 patients with SAD with the beta-blocker, atenolol, which has been shown to suppress melatonin secretion. We found that in the population as a whole there was no significant difference between the effects of melatonin and of placebo. However, some patients appeared to do extremely well on atenolol throughout the winter months. These patients relapsed when atenolol was discontinued and responded once again when it was reinstated. It is possible that there is a subgroup of patients with SAD in whom the secretion of melatonin plays an important role in the pathogenesis of their symptoms. The study of skeleton photoperiod treatments in 7 patients with SAD (see report number Z01 MH 02205-03 CP) also suggests that melatonin secretion may not play a major role in the pathogenesis of SAD in most patients. Naturalistic studies of plasma melatonin profiles over a period of 48 hours have been conducted in 7 SAD patients and 7 normals in both summer and winter and these samples await analysis.

It is not clear at this time what role melatonin secretion has in the pathogenesis of SAD or in the response of this condition to phototherapy. Some of our studies suggest that melatonin has some role in these processes, at least in certain patients. However, all studies argue against a major role for melatonin in the population as a whole and the skeleton photoperiod study, which is perhaps the most clearly negative one, raises the possibility that melatonin secretion has no role at all.

Project Description:

Seasonal affective disorder (SAD) is a condition characterized by recurrent winter depressions interspersed with periods of euthymia or hypomania in the spring and summer. The seasonal variation in behavior which is typical of this condition resembles the seasonal rhythms which are widely prevalent among other animals. These animal seasonal rhythms are frequently influenced by naturally occurring time cues, of which photoperiod (the illuminated portion of the 24-hour day) is by far the most important. Manipulations of photoperiod in the laboratory have been shown to be capable of modifying seasonal rhythms in a variety of animals. In studying the neurochemical mediation of these photoperiodic effects, the secretion of the pineal hormone, melatonin, has almost invariably been shown to play a key role. Melatonin is secreted at night in a circadian rhythm generated by the suprachiasmatic nuclei (SCN) of the hypothalamus. The timing of photoperiod influences melatonin secretion by means of a neuroanatomical pathway extending from the retina, via the retinohypothalamic tract, the SCN, and the superior cervical ganglia to the pineal gland. Photoperiodic information acts by means of a direct suppressing effect on the secretion of melatonin as well as by an entraining effect, influencing the timing of the rise and fall of melatonin.

The key role of melatonin has been established in laboratory animals by exogenously administering this substance and showing that the timing of such exogenous administration influences can be the sole determinant of the seasonal behavior being studied and can override the influence of photoperiod. In patients with SAD we have shown that extension of the photoperiod with bright (2500 lux) environmental light is capable of reversing the symptoms of SAD. Light of this intensity can suppress the secretion of melatonin in humans whereas light of ordinary room light intensity cannot. We have shown that ordinary room light cannot reverse the symptoms of SAD in most patients. These findings, together with the importance of melatonin as a mediator of seasonal rhythms in animals, led us to explore the role of melatonin as a mediator of the symptoms of SAD and the therapeutic effects of phototherapy. The year before last we administered melatonin exogenously to 8 patients with SAD, who had been previously treated with phototherapy, and found that melatonin reinduced some but not all the symptoms of SAD. This past year we have extended our studies.

Methods:

Patients were recruited via the media and community referrals. They were screened for a history of SAD and were followed clinically from summer into winter. When they became depressed [Hamilton Rating Scale (HRS) > 13], they were randomly assigned to one of two treatments, atenolol or placebo, for a period of one week. They then entered a week of withdrawal from the first treatment, which was followed by a week of the alternate treatment. The rationale for this study is that atenolol, a beta-adrenergic blocking agent, is known to block the secretion of melatonin,

which is occurs under normal physiological conditions as a result of stimulation of pineal beta-adrenergic receptors. HRS scores were recorded at the end of each week of treatment and withdrawal by raters who were blind to the patients' treatment condition as were the patients themselves. Twenty-four hour urine samples were collected from patients under both placebo and atenolol conditions and the excretion of the major melatonin metabolite, 6-hydroxy-melatonin-sulphate (6-OH-MS) was measured by radio-immunoassay.

Findings to date:

Eighteen SAD patients were treated with atenolol and placebo. Although a significantly lower amount of 6-OH-MS was secreted in the urine under atenolol than under placebo conditions, there was no statistical difference between the response to atenolol and to placebo. However, certain patients seemed to respond especially well to atenolol, relapsed when atenolol was withdrawn and responded once again when it was reinstated. These patients were maintained on atenolol throughout the winter months to good effect.

Significance to Biomedical Research and to the Program of the Institute:

Having previously described the syndrome of SAD and an effective new treatment for this condition, namely phototherapy, we have now made further contributions to the understanding of this condition by beginning to define the role of melatonin in this disorder. In view of the animal literature melatonin appeared to be the likeliest candidate as a mediator of the condition and the effects of phototherapy. The finding of this study, taken together with the findings of other studies, listed in the other annual reports on this condition, suggest that melatonin, while perhaps of some importance in some patients with SAD, is probably not of pivotal importance in the pathophysiology of most cases of SAD and in the mediation of most responses to phototherapy. While the "melatonin hypothesis" has not completely been laid to rest, the finding of the above study suggests that researchers should look elsewhere for the answers to the above questions.

Proposed Course:

Our proposed course depends in part on the results of the analysis of our plasma samples taken from patients both under naturalistic conditions and following a challenge with the melatonin precursor, 5-hydroxymelatonin (see annual report number Z01 MH 02206-02 CP). Assuming these results do not reveal an abnormal pattern of melatonin secretion in patients with SAD, this would discourage further studies in this area. However, in view of the partially positive results at least in some patients, the role of melatonin in SAD should probably continue to be investigated. One study which we may undertake is to administer melatonin to SAD patients in the fall months when they have not as yet developed the symptoms of SAD but when they are presumably vulnerable to doing so. It would be important to administer melatonin to normal volunteers as well to ascertain

whether melatonin is eliciting a specific vulnerability or whether the behavioral effects are simply the function of the non-specific sedation described by others.

Perhaps the most important outcome of the above study, as well as its companion studies mentioned in the other annual reports, is that it focusses attention on the need to study other possible neurochemical mediators of seasonal effects and the response to phototherapy in patients with SAD. We intend to so in the coming year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02222-02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Treatment of Rapid-Cycling Manic-Depressive with Thyroxine Illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. A. Sack	Chief, Inpatient Services	CPB/NIMH
Others:	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
	N. E. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	B. L. Parry	Clinical Associate	CPB/NIMH
	W. B. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	S. P. James	Clinical Associate	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Disturbances in the hypothalamic-pituitary-thyroid axis (HPT) may have an etiologic role in patients with rapid-cycling manic-depressive illness. These patients are more likely to develop lithium-induced hypothyroidism and in open clinical trials their mood fluctuations may improve when treated with hypermetabolic doses of thyroxine. The purpose of our present study is to define the nature of the HPT abnormality in these patients to assess in a double blind, placebo controlled trial whether thyroxine is an effective treatment in these patients.

As part of ongoing research we have studied the therapeutic effects of euthyroid and hypermetabolic doses of thyroxine in six patients with a history of rapid-cycling manic-depressive illness. Of the six, five completed the study. All subjects were treated with euthyroid and hypermetabolic doses of thyroxine.

Two patients with extremely short cycles (6-8 days) remitted when treated with hypermetabolic doses and relapsed when their dose of thyroxine was decreased. Of the other three patients, two were antidepressant-induced cyclers, who were depressed but not cycling at the time of the study. Neither of these patients showed any improvement.

Our preliminary results suggest that: 1) hypermetabolic but not euthyroid treatment with thyroxine may be effective in treating rapid cyclers 2) patients with very short cycles which are not drug-induced are most likely to benefit from this treatment.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

This project is designed to assess the hypothalamic-pituitary-thyroid axis (H.P.T) in rapid-cycling manic-depressives and to test the therapeutic efficacy of exogenously administered thyroxine in these patients. A complete description of the project and methods appears in Z01 MH 02222-01 CP.

Results:

In the last year we have completed the study of the circadian rhythm of T.S.H. in nine rapid-cycling manic-depressives and nine age and sex-matched normal controls. The rhythm for TSH is ordinarily highest at night, but the magnitude of the nocturnal rise is attenuated by sleep. When normals are kept awake at night (sleep deprived) a significantly greater rise in TSH occurs. All patients except one, and all nine controls, were studied for a 42-hour period with samples drawn every 30 minutes for TSH and cortisol, which included a night of sleep deprivation on the second night.

An ANOVA with repeated measures was performed on the TSH and cortisol data. TSH was significantly lower in the patient group both at baseline and on the sleep deprived night. Paired t-tests revealed significant patient-control differences at 11:30 p.m., 1:30 a.m., 5:30 a.m., and 6:30 a.m. but no differences occurred during the daytime, sleep deprivation was associated with higher nocturnal TSH in both groups, but this effect was more marked in the normal controls. With sleep deprivation the TSH levels in the depressed group approximated the levels seen in the normal controls at baseline. Cortisol secretion did not differ between patients and normal controls and there was no significant effect of sleep deprivation on cortisol secretion.

This study suggests that in rapid-cycling manic-depressive illness there is a dysregulation of TSH which is present at baseline, which was not observed in earlier reports which examined only daytime values. The normalization of TSH with sleep deprivation suggests that the antidepressant effects of sleep deprivation may be related to stimulation of the H.P.T. axis.

As part of ongoing research we have studied the mood stabilizing effects of thyroxine in six patients with rapid-cycling manic depressive illness in a double blind controlled trial. Rapid cycling was defined as a history of at least four or more manic or depressive episodes in one year at some time in the course of illness. It is recognized that in a subgroup of rapid cyclers, the cause is iatrogenic, i.e. induced by treatment with tricyclic antidepressants. These patients will stop cycling but remain fixed in their depression when the antidepressant medications are withdrawn. In our study we have included both drug induced as well as spontaneous rapid cyclers. All patients were treated with replacement doses of thyroxine first. After a period of at least 4 weeks they were randomized to either additional therapy with replacement doses or to treatment with hypermetabolic doses of thyroxine. Thyroxine was increased at weekly intervals until levels of T_4 were approximately 100% greater than at baseline.

Of the six subjects treated, five completed the trial. The sixth was dropped from the study because of an exacerbation of anxiety symptoms which occurred when the thyroxine was increased into the hypermetabolic range. In two subjects a sustained improvement in mood was noted with hypermetabolic but not euthyroid treatments. At the end of the trial their doses of thyroxine were decreased to the euthyroid range. In both cases the patients relapsed and clinical improvement was noted when the thyroxine was reinstated at the higher dose. Both of these subjects had extremely short cycles, approximately 6-8 days, and their cycles occurred spontaneously.

None of the remaining three subjects improved. However, two of these subjects were in a continuously depressed state throughout the trial, their cycles having ceased when their antidepressant medication was withdrawn.

If confirmed with a larger sample, our work indicates that: 1) hypermetabolic doses are required for the therapeutic response, thyroxine, 2) only patients with spontaneous cycles are likely to respond to thyroxine treatments; 3) patients with prominent anxiety symptoms may be unable to tolerate treatment with thyroxine.

In the last year we have completed the study of the circadian rhythm of TSH in nine rapid-cycling manic-depressives and nine age and sex-matched normal controls. The rhythm for TSH is ordinarily highest at night, but the magnitude of the nocturnal rise is attentuated by sleep. When normals are kept awake at night (sleep deprived) a significantly greater rise in TSH occurs. All patients except one, and all nine controls, were studied for a 42-hour period with samples drawn every 30 minutes for TSH and cortisol, which included a night of sleep deprivation on the second night.

Significance to Biomedical Research and to the Program of the Institute:

1. Approximately 15% of all manic-depressives respond poorly to lithium carbonate and of these the majority are rapid-cyclers. These patients constitute a large refractory group for whom present treatments are inadequate.

2. Rapid cyclers demonstrate a higher incidence of thyroid abnormalities than other bipolar patients. The anecdotal literature suggests that rapid cyclers may respond to treatment with thyroxine whereas non-rapid cycling bipolars do not improve with thyroxine alone. Thus, these patients provide a model in which to study physiological interactions between thyroxine and neurotransmitters on mood.

Proposed Course:

Over the next year we intend to study an additional six rapid cycling patients. We will also study the circadian rhythm of TSH in non-rapid-cycling patients in order to assess the specificity of this finding. Since the physiologic basis for the circadian rhythm of TSH is not known, future studies will be directed at assessing the role of TRH in this rhythm, and exploring the suppressions effects of sleep on TSH.

The fact that increased TSH is associated with sleep deprivation suggests that TRH administered at night might be therapeutic in depression. We are planning to study this in the near future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02223-02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pentobarbital and Ethanol Toxicity: Relation to the Benzodiazepine Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:	J. V. Martin	Staff Fellow	CPB/NIMH
	R. Wagner	Guest Worker	CPB/NIMH

COOPERATING UNITS *(if any)*

LBC/NIADDK
Rockland Research Institute

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.3	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because of reports that both barbiturates and ethanol interact with the GABA-benzodiazepine-chloride channel complex, we embarked on a series of studies to determine if agents which block various aspects of the complex might reduce toxicity from high doses of these sedatives. We reported last year that IPPO, a chloride channel blocker, greatly reduced deaths from pentobarbital (PB) overdose and reduced loss of righting reflex from ethanol. We have continued these studies looking for agents which are both more effective, and less toxic when given by themselves. We found that TBPS, also a chloride channel blocker, was equally effective as IPPO but had less toxicity. We are currently investigating adenosine antagonists, in this same paradigm.

Project Description:

Please refer to Z01 MH 02223-01 CP.

Methods:

Rats were treated with pentobarbital or ethanol followed by a variety of agents which lead to various components of the BZ receptor complex, as seen in the attached table.

Findings to Date:

TBPS, at a dose of 244 lowered mortality from PB from virtually 100% to 60%. TBPS given by itself had a lethality of 15%.

Significance to Biomedical Research and to the Program of the Institute:

The perturbation of the GABA-benzodiazepine receptor-chloride ionophore complex by PB and ethanol *in vitro* suggests that some of the pharmacologic properties of these agents could be mediated at these sites. These *in vitro* studies are consistent with *in vivo* evidence that picrotoxin and CGS 8216 may reverse some actions of PB. This led to the present study in which the effects of a series of ligands binding to various components of the supramolecular complex were examined for their abilities to reverse PB and ethanol toxicities. It was found that IPPO, which binds to the chloride ionophore, can partially reverse the toxic effects of pentobarbital in a dose-dependent manner. Picrotoxin, which presumably binds at the same site and has been used clinically in barbiturate poisoning, can also partially prevent pentobarbital lethality. However, at equieffective doses, picrotoxin is significantly more toxic than IPPO and, in contrast to IPPO, shortens the latency to death. Two other agents that bind to the dihydropicrotoxinin site, MT 11 and cartazolate, had no effect on PB mortality. These findings suggest that binding at or near the chloride ionophore per se is not sufficient, and that protective effects are dependent on a more subtle interaction of ligand and receptor. Strychnine does not affect pentobarbital-induced mortality, suggesting that the effects of IPPO are specific and not common to all convulsants. Further, IPPO did not prevent ketamine mortality, suggesting that its effects may be restricted to depressants which are ligands at the chloride ionophore site (or supramolecular complex). A dose-dependent effect on ethanol-induced LRR was also seen with IPPO. These observations suggest that some aspects of the toxic effects of pentobarbital and ethanol may be reversed by ligands which bind to the supramolecular complex.

Although the toxicity of IPPO is substantially less than that of picrotoxin, its clinical usefulness appears limited. However, our results clearly show that various ligands which bind to the dihydropicrotoxinin site can have widely varying potencies in protection from pentobarbital mortality. Further, the potencies are not necessarily correlated with the toxic effects of the ligands given alone. These observations suggest that it would be fruitful to use this approach to find a related compound for the treatment of toxicity from barbiturates or ethanol.

Proposed Course:

We are continuing to evaluate a series of compounds which act at the receptor complex in order to find one which is even more effective and more benign when given alone.

Summary of Experimental Design and Results

Injection 1[1,2]	Injection 2	% Seizures with Vehicle as Injection 1	% Deaths with[3] Vehicle as Injection 1	% Deaths with[4] Active Drugs as Injection 1
PB or Vehicle	125-500 μ/kg IPPO or vehicle	8-80%	0-45%	58-8%
PB or Vehicle	4-16 mg/kg picrotoxin or vehicle	20-92%	0-90%	58-95%
PB or Vehicle	1.5-6 mg/kg strychnine or vehicle	30-75%	20-50%	100%
PB or Vehicle	7.5-30 mg/kg R5135 or vehicle	20-85%	0-90%	95-100%
PB or Vehicle	40-240 mg/kg MT 11 or vehicle	0-60%	0-15%	92-100%
PB or Vehicle	20-80 mg/kg Cartazolate or vehicle	0-55%	0-5%	80-90%
PB or Vehicle	5-20 mg/kg CGS 8216 or vehicle	0	0	80-98%
PB or Vehicle	30-60 mg/kg @CCT or vehicle	0	0	75-95%
Ketamine or Vehicle	125-500 mg/kg IPPO or vehicle	0-95%	0-55%	80-100%

[1]Injection 1 was followed 5 min. later by Injection 2. Death latencies are measured from time of injection 1.

[2]The dose of pentobarbital was always 170 mg/kg. The dose of ketamine was 300 mg/kg.

[3]In those animals who received vehicle for both injections, there were no deaths.

[4]In each experiment, a group of mice were given active drug as injection 1, followed by the vehicle for the drug given at the second injection. Pentobarbital caused an average of 94 $\pm$ 4% deaths (range=75-100%). The dose of ketamine used caused 80% of the mice to die.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02224-02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Dihydropyridines on Benzodiazepine-Induced Alterations in Ca^{2+} flux

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:	J. V. Martin	Staff Fellow	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The in vitro findings described for this project, i.e., that diazepam increases calcium uptake into synaptosomes and that this may be blocked by nifedipine, have led to a series of in vivo sleep studies (Project Z01 MH 02225-02 CP). We continue to be engaged in the sleep studies which came from this biochemical work, but have not needed further biochemical analyses of this response during the past year. This project number is, then, terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02225-02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies in the Role of Calcium Flux in the Sleep-Inducing Effects on Flurazepam

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:	J. V. Martin	Staff Fellow	CPB/NIMH
	R. Wagner	Guest Worker	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.3	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous studies have indicated that there may be a functional relationship between benzodiazepine (BZ) receptor stimulation and alterations in calcium flux. We reported last year, for instance, that nifedipine, a calcium channel blocker, would prevent the sleep-inducing effects of flurazepam in rats. We have shown that BAY K 8644, a calcium channel agonist, enhances the sleep-inducing properties of flurazepam.

Project Description:

It is now well established that the anxiolytic, anticonvulsant and muscle relaxant properties of the benzodiazepines are mediated by interaction with high-affinity, stereospecific receptors. Recent work from this laboratory suggests that this is also true for the sleep-inducing properties, insofar as low doses of 3-hydroxymethyl-B-carboline, which binds to these receptors, prevents sleep-induction by flurazepam. The mechanism by which receptor stimulation leads to pharmacologic effect has not been established. Although It is generally well accepted that benzodiazepines potentiate GABA-mediated chloride conductance, other ionic effects have also been proposed. There have been reports that benzodiazepines enhance calcium entry into synaptosomal preparations, an observation compatible with the hypothesis that altered calcium flux may be involved in the actions of benzodiazepines. Recent studies from our laboratories have shown that pharmacologically relevant concentrations of benzodiazepines selectively enhance the potassium-depolarized uptake of calcium into cerebral cortical synaptosomes. These effects are blocked by the benzodiazepine antagonist CGS 8216 and the GABA antagonist bicuculline. Administration of calcitonin (which lowers serum calcium concentrations) has been reported to decrease sleep in humans. These observations led us to examine the interaction between nifedipine, a calcium channel blocker, and flurazepam on sleep in the rat.

Methods:

Animals were administered vehicle or 100 μg/kg BAY K 8644 intraventricularly, followed by vehicle or 40 mg/kg flurazepam IP. Standard two-hour sleep recordings were then performed.

Findings to Date:

As expected, flurazepam lowered sleep latency from control values of 22.4 $\pm$ 2.4 min to 17.2 $\pm$ 1.6 min BAY K 8644, which had no effect on sleep latency by itself (24.8 $\pm$ 3.8 min), led to a further enhancement of the reduction in sleep latency by flurazepam (6.9 $\pm$ 0.72 min).

Significance to Biomedical Research and to the Program of the Institute:

It has been previously reported that benzodiazepines enhance calcium uptake into synaptosomes, and this effect is blocked by the benzodiazepine receptor antagonist CGS 8216. These observations are consistent with the hypothesis that calcium ion flux is part of the effector mechanism subsequent to occupation of benzodiazepine receptors. This hypothesis is further supported by the recent finding that nifedipine prevents the diazepam-induced increase in synaptosomal calcium uptake. The present data may further demonstrate a specificity in function of the calcium-mediated aspect of benzodiazepine action. Calcium ions may be more directly involved in sleep regulation thatn in other effects of benzodiazepines.

The exact site of action of calcium channel blockers such as nifedipine in the brain is not yet established. Further study of the relationship of

benzodiazepines and calcium flux may aid in elucidating the mechanism of action of these widely used agents.

Proposed Course:

Studies are currently under way to assess the possible role of calcium flux in non-benzodiazepine hypnotics and anticonvulsants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02226-02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relation of Rhythms of Core Temperature and Sleep

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:	N. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	D. Sack	Chief, Inpatient Services	CPB/NIMH
	T. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS *(if any)*

BEIB/NIH

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Preliminary data from this study indicated that despite the application of heating and cooling at the appropriate times through a network of tubing surrounding the subject that a circadian rhythm of temperature largely remained intact. For this reason, we have terminated further work on this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02227-02 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insomnia: daytime and nighttime functioning

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:			
	M. Linnoila	Clinical Director	DICBR/NIAAA

COOPERATING UNITS *(if any)*

LPP/NIMH
LCS/NIAAA

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.3	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has merged to become one aspect of project Z01 MH 02193-03 CP.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02290-01 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Melatonin Analysis in Clinical Blood Samples

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
Others:	S. Shapiro	Chemist	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of melatonin in blood has been controversal; many assays exist and there is little quantitative agreement among laboratories. In our laboratory, we found that our quantitative determination of melatonin in plasma was not consistent with our previous observations. After extensive re-assessment of the assay, we found that alteration in pH of one wash solution altered, by 2 orders of magnitude, the quantitative estimation of the concentration of plasma melatonin.

Project Description:

The Branch's clinical program has been based on hypotheses that required analysis of circulating melatonin. The radioimmunoassay of Rollag and Niswender, which had been validated for use in human plasma by HPLC by our laboratory, was the method of choice. However, due to technical changes in the water and buffers supplied to us by the NIH media unit, we found significant interference for the antibody - ligand binding in extracted and washed plasma samples.

The ability of the ligand to be competitively displaced by cold melatonin indicated that the antibody-ligand interaction was still valid. However, the matrix for our plasma had been altered and was no longer satisfactory for measurement of melatonin. We have now successfully resolved this problem and have, in fact, lowered our estimation of daytime levels of melatonin in humans.

Methods:

Human plasma was extracted with 4 volumes of chloraform. The plasma was aspirated and the chloroform washed with 1M HCO_3, pH 8.4; 1M HCO_3 pH 10.25; 1N NaOH or 2N NaOH. This layer was aspirated and distilled water was added. An aliquot of the chloroform was removed, evaporated to dryness, the sample reconstituted and extracted with 5 volumes of petroleum ether. An aliquot of the aqueous layer was removed and the antibody and ^{125} I-melatonin analog added. The assay was incubated for 48 hours and the bound was separated from the free by precipitation with 3 cc cold ethanol.

Results:

Using the same aliquot of human plasma in the same assay the quantitative analysis following washing with 1M HCO_3, pH 8.4 was 86 pg/ml, with 1M HCO_3, pH 10.25 was 28 pg/ml, with 1N NaOH was 2 pg/ml, and with 2N NaOH was 8 pg/ml. Clearly, this one washing procedure significantly altered quantitative analysis of melatonin in this assay. Quantitative recovery of cold melatonin added to this human plasma sampled revealed that 83% of the added melatonin was observed upon analysis. These data indicate that 1N NaOH washing reduces melatonin immunoreactivity and does not destroy added melatonin. These modifications have improved our analysis of clinical samples and assisted laboratories in Chicago, Brussels, and Ann Arbor to perform this analysis better.

Significance to Biomedical Research and to the Program of the Institute:

Resolving analytical controversy by improving the method of melatonin analysis provides the field with a reliable way to measure samples and to address biologically meaningful questions. Continued controversy only detracts from the major scientific efforts in the field and obscures significant issues in melatonin clinical research. By making this information immediately available, we have shown that our mission is to promote high quality research in an atmosphere of sincere cooperation.

Proposed Course:

We plan to make available our findings by forwarding our modifications of the assay with every request for antibody. We hope this will reach all those concerned, to avoid any further delay in changing analytical procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02291-01 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Site of Action of Melatonin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
Others:	S. Shapiro	Chemist	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using two model systems investigations of melatonin's possible intracellular action were assessed. In vitro, MCF-7 human breast cancer cells were chosen and in vivo, uteri from ovariectomized hamsters were used. Melatonin had previously been shown by us to have an acute effect on estrogen receptors in both systems, and one hypothesized modification of estrogen receptors is their phosphoylation to an active form. This phosphorylation might be regulated by cAMP dependent protein kinase, suggesting an effect of melatonin on cAMP concentrations, or cGMP concentrations, or on cAMP-dependent protein kinase. We have investigated melatonin's effect on these three parameters in these two model systems.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Studies have been conducted to determine melatonin's mechanism of action. Using an *in vitro* cell system (MCF-7 cells) we have attempted to determine melatonin specific binding in a whole cell assay and in a cytosolic assay. This approach is similar to that used to describe steroid receptors, and the chemical similarity of melatonin to steroids suggested this line of investigation. The lack of melatonin-specific binding in this cell line which appears responsive to melatonin treatment led me to the conclusion that the mechanism of melatonin's action may not be similar to the well described models for hormone action. An alternative approach could be that melatonin may affect protein phosphorylation, which would be consistent with its subtle effects *in vitro* and *in vivo*. One mechanism for the regulation of protein phosphorylation is through the cyclic nucleotides, cAMP and cGMP, and the cAMP-dependent protein kinases. Changes in these parameters were investigated *in vivo* and *in vitro*.

Methods:

I. Melatonin binding assays

A. Whole cell assay

MCF-7 cells were grown to confluence in T-75 flasks, media removed, and replaced with phosphate buffered saline (PBS) containing 2nM ^{125}I-melatonin analog (approximately $4x10^6$ cpm/ml). After 40 minutes incubation the cells were washed with PBS and then the cells were lysed with 4ml of 70% ethanol. A 500ul aliquot was removed and counted in a gamma counter.

B. Cytosol assay

MCF-7 cells were grown to confluence in T-75 flasks and the cells were detached using typsin-EDTA. The 5 ml aliquot of cells was then subjected to sonic treatment to disrupt the cells. The homegenate was spun at 25,000 rpm and the supernatant was harvested. Aliquots (500 ul) of cytosol were incubated with varying concentrations of ^{125}I-melatonin analog with or without 1000-fold excess of melatonin.

II. cAMP and cGMP assays

cAMP and cGMP were analyzed in cytosol prepared from MCF-7 cells or ovariectomized hamster uteri by commerically available kits (New England Nuclear).

III. cAMP-dependent protein kinase assay

These protein kinases specifically bind cAMP. Using an azido containing, ^{32}P labeled cAMP we incubated this molecule with cytosol prepared from MCF-7 cell treated with or without melatonin or uteri from ovariectomized hamsters treated with or without melatonin. The MCF-7 cells were treated either acutely (60 min) or chronically (2 to 4 days) with

melatonin. Hamsters were given a single 25 ug injection of melatonin and sacrificed 40 minutes later.

After preparing cytosol from these tissues the azido, ^{32}P labeled cAMP was incubated with or without 1000-fold excess of cAMP. After a 60 minute incubation the mixture was exposed to 3 minutes of UV light. This results in the covalent binding of receptor bound azido, ^{32}P-cAMP. The mixture is heated and the denatured protein separated on 10% polyacrylimide gel by electrophoresis. For each test cytosol the azido, ^{32}P-cAMP was incubated with or without excess unlabeled cAMP to identify specific cAMP binding to protein. The resultant gel was placed against X-ray film and after 1 day exposure specific protein bonds containing ^{32}P-cAMP were identified.

Findings to date:

I. Melatonin binding studies

Using a whole cell assay system we were unable to determine a significant amount of binding. One limitation of the ^{125}I-melatonin analog is its polarity which may preclude its entry into the cell. Using ^{3}H-melatonin which would have access to the intracellular compartments, the specific activity is too low to truly determine specific binding. Although this method is the most gentle, physiologic way to look for specific uptakes of melatonin, it appears to have significant limitations which question its usefulness.

Using the cytosol preparation ^{125}I-melatonin analog was bound to some cellular fraction. By varying the concentration of ^{125}I-melatonin analog proportionately the same amount of ligand was bound. At these various concentrations of radioactive ligand the excess unlabelled melatonin was unable to displace the bound ligand. This suggests that the ligand binding observed was non-specific and may not be associated with melatonin-specific binding.

II. Effect of melatonin on cAMP and cGMP concentrations

Although melatonin appeared to alter cAMP levels in cytosol from MCF-7 cells, repeated studies failed to confirm this initial observation. cGMP levels were not different in melatonin treated versus untreated cells. cAMP levels were not different in the cytosol of uteri from melatonin treated or untreated hamsters.

III. Effect of melatonin on cAMP-dependent protein kinase

Using this photoaffinity labeling technique, we were able to visualize specific cAMP binding to intracellular proteins. The molecular weights of these proteins correspond to that reported by others to represent type I kinase (43,000 daltons), type II kinase (54,000 daltons) and the type II breakdown product (36,000 daltons). Additionally, these bands on the gel were absent when the samples had 1000-fold excess of cold cAMP added.

Qualitatively, melatonin may have altered the amount of type I kinase. However, this effect has thus far not been consistently observed and further experiments need be done to determine if melatonin could effect protein phosphorylation through the cAMP-dependent protein kinase system.

Significance to Biomedical Research and to the Program of the Institute:

The mechanism of action of the hormone melatonin remains a mystery. We have demonstrated that it has biologic action, but that it does not conform to known mechanisms of hormone action. Unraveling this problem will potentially provide us with new insight into hormone action and will allow us to ask the question of how melatonin acts centrally.

Proposed Course:

We will continue to evaluate melatonin's role in protein phosphorylation. In addition to the already described cAMP-dependent protein kinase system, we will evaluate melatonin's effect on protein kinase C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02292-01 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Melatonin Effect on Hormone-stimulated Cell Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
Others:	S. Shapiro	Chemist	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Physiologic concentrations of melatonin have been previously shown by us to increase estrogen receptor concentrations in vitro in a human breast cancer cell line. This in vitro model system is being investigated further to determine if this will be a reliable biological response and to assess what hormonal parameters affect the melatonin response of these cells to melatonin. The present data indicate that melatonin promotes estrogen-stimulated growth of MCF-7 cells implanted in nude mice and promotes tumor growth in vitro of insulin-stimulated MCF-7 cells.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

In previous investigations we have found that melatonin may play a role in the etiology of breast cancer in rats and humans. In rats, melatonin can inhibit while pinealectomy can enhance the incidence of dimethylbenzanthracene-induced mammary tumors. In humans, the concentration of estogen receptors in each patient's tumor was inversely correlated with the nocturnal level of melatonin; suggesting that lower nocturnal levels of melatonin are correlated with increased incidence of hormone dependent breast cancer.

In vitro in a hormone dependent breast cancer cell line (MCF-7) we have found that melatonin will induce an increase in estrogen receptor concentration acutely, within 40 minutes. This increase in estrogen receptors is apparently contradictory to the long-term effect of melatonin *in vivo*. To resolve this we are using two approaches: 1) determine the effect of melatonin on MCF-7 cell growth in cell culture, and 2) determine effect of melatonin on tumorigenesis in nude mice injected subcutaneously with MCF-7 cells.

Methods:

MCF-7 cells are propagated in improved minimal essential media, supplemented with 10% fetal calf serum and insulin to insure continued growth of hormone dependent cells. For the *in vitro* growth study, a minimal number of cells are plated in wells and media containing 0, 10^{-9}, 10^{-7}, 10^{-5} M melatonin. After two days and continuing for another 5 days cells are detached from the wells using trypsin and EDTA and counted in a hemocytometer. For the *in vivo* tumorigenesis study, athymic nude mice are injected with MCF-7 cells and implanted with estrogen "slow release" tablets. After 7 days all animals are checked for the presence of tumors. Half of the tumor containing animals are implanted with melatonin "slow-release" tablets. After 7 days all animals are checked for the presence of tumors. Half of the tumor containing animals are implanted with melatonin "slow-release" tablets. Weekly, the animals were checked and the tumors measured with caliphers. After 4 weeks the animals are sacrificed and tumors checked and measure with calipers.

Results:

Using our current methodology insulin-treated MCF-7 cells reach confluence in 7 days. During the 5 days of observation there were consistently 25-30% more cells in the melatonin treated wells, suggesting that melatonin enhanced the growth promoting effect of insulin. Similarly, nude mice treated with estrogen and melatonin had tumors that grew faster and larger than those treated with estrogen alone.

Significance to Biomedical Research and to the Program of the Institute:

This acute effect of melatonin provides us with an assay for the biology of melatonin. This response of melatonin in hormone dependent cancer cells suggest

that melatonin may play a role in the etiology of hormone dependent cancer and also provides us some insight into melatonin's mechanism of action. With an understanding of melatonin's effect in tumorigenesis, we will have the necessary tools to investigate its physiologic action, where all available data suggest that melatonin acts centally.

Proposed Course:

We plan to further substantiate these initial observations in these hormone dependent breast cancer cells. Our next step would then be to investigate other hormone dependent (human melanoma cell line) and non-hormone dependent cell lines for melatonin's effect on these. This approach would inform us of the specificity of melatonin's effect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02293-01 CP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Differential Response of the Uterus to Melatonin in Juvenile and Adult Syrian Hamsters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
Others:	S. Shapiro	Chemist	CPB/NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The reproductive effects of melatonin in the Syrian hamster may be due to its action upon steroid receptors in peripheral, pituitary or brain tissues. By examining the effect of melatonin in a peripheral target tissue the possible mechanisms for melatonin action may be less complex than that seen centrally. We have previously observed that melatonin caused a transient increase in estrogen receptors in uteri from juvenile hamsters. We now find that adult ovariectomized hamsters have decreased concentration of estrogen receptors following melatonin treatment. Further, ^{3}H-estradiol uptake was reduced in the uteri of melatonin treated adults and finally, melatonin partially inhibited estrogen-stimulated uterine growth. These observations suggest that melatonin affects estrogen action and this effect is dependent on the sexual development of the animal.

Project Description:

In a previous study we found that melatonin caused a transient increase in estrogen receptors in the uteri of juvenile Syrian hamsters, suggesting this to be one target for melatonin action. However, in adults melatonin appears to exert a negative effect on reproduction. We tested whether the positive effect of melatonin on estrogen receptors in juveniles would also be true in the adult. Our approach was to investigate melatonins's effect in ovariectomized adults to avoid the added confusion of the cyclic change in estrogen levels throughout the estrous cycle.

This project has taken three approaches: first, determining the effect of melatonin on estrogen receptors in adults compared to juveniles; second, evaluating specific ^{3}H-estradiol uptake in uteri from juvenile and adult animals; and third, assessing the effect of melatonin on estrogen-stimulated uterine growth. These data indicate that melatonin has positive effects on estrogen action in the juvenile, but negative effects in the adult.

Methods:

The concentration and affinity of uterine estrogen receptors was determined by a dextran-coated charcoal assay to compare the effects of melatonin on estrogen receptors in intact 20 day old juveniles and 45 day old adults (ovariectomized at 34 days of age). The hamsters received either 25 ug melatonin or saline sc at 1400 h (lights on 0100h to 1500h) 40 minutes prior to sacrifice. Uteri were excised and stored at -70° until analysis. Estrogen receptors were determined in the cytosol of these uteri. Cytosol was prepared by pulvarizing, homogenizing, and centrifuging at 100,000 xg for 1 hour. The resultant supernatant was incubated with varying doses of estradiol with or without excess DES overnight and the bound hormone was separated by the addition of dextran-coated charcoal. The resultant data were analyzed by computer analysis to determine receptor concentration and the dissociation constant.

A less traumatic method to evaluate melatonin's effect on uterine estrogen action was to simply inject animals with ^{3}H-estradiol and determine specific uptake in this tissue. Specifically, juvenile or adult ovariectomized animals were injected sc with either melatonin (25 ug), phosphate buffered saline, melatonin and diethylstilbesterol (60 ug) or phosphate buffered saline and diethylstilbesterol. 45 minutes later, all animals were injected sc with 60 ug ^{3}H-estradiol. After 90 minutes the animals were sacrificed and the uteri excised, cleaned of excess fat, washed with 2 ml of phosphate buffered saline and placed in 1 ml NCS tissue solubilizer overnight. 8 ml of scintillation fluid was added to each sample, and then counted in a scintillation counter. The specific ^{3}H-estradiol uptake was determined by taking the mean dpm of the diethylstilbesterol-treated groups and subtracting that from the mean dpm of the melatonin or phosphate buffered saline-treated groups.

To determine the physiologic effect of melatonin on the uterus of ovariectomized adult hamsters were treated with 10, 20 or 40 ug of

estradiol/day for 4 days at 0800h and saline or melatonin (25 ug/day) at 1400h. The fifth day the animals were sacrificed, uteri excised and weighed.

Findings to date:

The results from 11 separate experiments for juvenile hamsters and 14 separate adult ovariectomized experiments indicate that melatonin caused a 97% increase in juvenile uterine estrogen receptor concentration, while it caused a 33% decrease in adult ovariectomized uterine estrogen receptor concentration. Scatchard analysis revealed that the estrogen receptor affinity remained unchanged in both juvenile and adult ovariectomized group following melatonin treatment (Kd = 10^{-10} to 10^{-11} ML).

^{3}H-estradiol uptake studies from juvenile and adult ovariectomized animals treated with melatonin, phosphate buffered saline, melatonin and diethystiltesterol or phosphate buffered saline and diethylstiltesterol followed by a ^{3}H-estradiol injection 45 minutes later, showed a 27% increase in ^{3}H-estradiol uptake in the juveniles resulting from melatonin treatment. A 36% decrease in ^{3}H-estradiol uptake was observed in the adult ovariectomized animals treated with melatonin.

The uterine weight of ovariectomized adults was stimulated with 40ug 17-B estradiol by approximately seven times compared to the control animals. Animals treated with melatonin and estradiol had uterine weights that were 22% less than estradiol treated animals. In a second experiment melatonin inhibited estrogen stimulated uterine growth by 57%.

Significance to Biomedical Research and to the Program of the Institute:

The acute effect of melatonin on a biologic system has been difficult to demonstrate. This provides a simple, quick animal model system to investigate melatonin mechanism of action. Once we determine how melatonin acts in peripheral target tissues, then we would be in a more advantageous position to investigate its action centrally. Melatonin's role as an internal zeitgeber could be effectively studied with a clearer notion of how melatonin acts intracellularly.

Proposed Course:

This physiologic response to melatonin provides us with one biologic model system to investigate the intracellular action of melatonin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02294-01 CP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antidepressant Pharmacology of the Rodent Circadian System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. C. Duncan	Research Psychologist	CPB/NIMH
Others:	L. Tamarkin	Research Biologist	CPB/NIMH
	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS *(if any)*

University of Maryland

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the major features of affective illness is disruption of the circadian sleep-wake cycle characterized by loss of sleep, intermittent and early morning wakefulness. Accompanying this disturbance are abnormalities in the temporal relationship of physiological processes such as hormone release and temperature regulation with the sleep-wake cycle. One prediction of the phase advance hypothesis of affective illness is that re-establishment of more normal temporal phase relationships between disordered circadian rhythms may result in remission of depressed symptomotology. The mechanism of antidepressant chemical treatments may be consistent with this prediction; previous reports indicate the psychoactive medications lithium and the MAO inhibitor clorgyline affect the mammalian circadian system. We have therefore begun to evaluate antidepressant chemical effects on the rodent circadian system. This project has necessitated the design, construction, and evaluation of a rodent circadian rhythm monitoring facility. In addition, we have recently completed preliminary experiments designed to clarify clorgyline's precise effect on the rodent circadian system.

Our studies to date demonstrate the rodent circadian facility is performing as designed; animals exhibit circadian patterns similar to those in controlled circadian laboratories elsewhere. Our pharmacological studies indicate a) direct or indirect clorgyline input to the hamster circadian pacemaker and b) clorgyline modification of the hamster rest-activity cycle. While this evidence is preliminary, we feel these data are currently the most compelling evidence of antidepressant chemical input to the rodent circadian pacemaker. Our current research plans are to replicate these findings and extend our observations to determine the mechanism and site(s) of clorgyline's input to the circadian system.

Project Description:

The circadian rhythm disturbances observed in depressed patients may be due to dysfunctional primary or secondary oscillators within the human circadian system. Effective antidepressant chemical treatments may affect mechanisms which correct these abnormalities. Therefore we are interested in exploring the actions of antidepressant drug treatments on the circadian system. This project's objectives were to 1) establish and evaluate a rodent circadian motor activity monitoring facility within the Clinical Center and 2) evaluate the effect of the antidepressant clorgyline on the rodent circadian system.

Methods:

1) Facility description and evaluation:

The temporally isolated rodent circadian facility consists of three major elements: a) An automated 11/23 data acquisition system capable of on-line collection of motor activity and environmental lighting from seventy-two individually housed hamsters. Data is stored on 10.4 MB Winchester disk and transferred to double density floppy disks. An uninterruptable power supply provides a constant power source in the event of local power failure b) Twelve independent, sound attenuated, light controlled, experimental chambers, each capable of individual housing for six hamsters in polycarbonate containers equipped with running wheels connected to a cam activated microswitch. Food and water are provided to animals ad lib for periods exceeding one month without experimenter intervention. Each chamber is ventilated with a high performance Muffin XL fan which exchanges air through intake and exhaust light baffles. A wall mounted, programmable Chrontrol microprocessor controls light-dark schedules independently in each of the twelve experimental chambers. c) A rodent colony maintained in similar light controlled chambers which permits precise investigator control over pre-experimental conditions, a major requirement in circadian research.

Evaluation of this facility indicates achievement of those conditions essential to conduct controlled circadian rhythm research. We have observed: 1) entrainment of motor activity rhythms by the light-dark schedule 2) freerunning motor activity rhythms in constant conditions and 3) no evidence of rhythm dissociation or relative entrainment in constant conditions. Utilization of this facility accelerates and expands our research capabilities.

2) Antidepressant drug effects on the hamster circadian system:

The response of the hamster circadian system to the MAOI clorgyline has been tested in two conditions a) during freerunning in constant darkness and b) during entrainment to a LD schedule. In both paradigms clorgyline (2 mg. $kg^{-1} \cdot day^{-1}$) or a saline control was administered chronically by subcutaneous implant of two-week miniature Alzet osmotic pumps. Pumps were implanted in the middle of the hamsters' inactive circadian phase with the assistance of red lighting when appropriate. Implants conducted in constant darkness were performed following 3-4 weeks of free-running activity rhythm. Pumps were removed following two weeks to document release of their contents.

Findings to Date:

In constant conditions, chronic, systemic clorgyline treatment resulted in a) a clear lengthening of the circadian motor activity period in four of five treated animals as determined by chi-square periodogram analysis, when compared to pre-treatment free-runs. The fifth animal exhibited a phase shift of the motor activity rhythm, b) a decrease in the amount of motor activity and c) an increase in the duration of the active portion of the circadian rest-activity cycle.

Following clorgyline implants in entrained animals we observed a) an immediate phase-delay in the onset of activity relative to the LD schedule, b) a general decrease in amount of activity, c) a change in the expression of motor activity characterized by a decrease in the amount of activity normally observed during the early active phase and an increase in the amount of activity normally observed during the late active phase, and d) following pump removal, transient phase-advance of the circadian motor activity rhythm.

Significance to Biomedical Research and to the Program of the Institute:

These preliminary experiments are the first to strongly indicate an antidepressant drug influences the frequency of the mammalian circadian pacemaker. We interpret these findings as evidence for clorgyline affecting either a) an input pathway or b) a gear of the oscillating mechanism itself.

The effects of clorgyline on the mammalian circadian pacemaker are consistent with predictions based on the phase-advance hypothesis of affective illness, specifically, chemical treatments which slow a putatively fast circadian oscillator in certain depressed patients are effective in treating depression.

The utility of the rodent circadian rhythm facility in evaluating the mechanism(s) of known antidepressant drug therapies and also the therapeutic potential of newly developed antidepressant medications from a chrono-pharmacological perspective is a significant development within the prgoram. Additionally, we anticipate this facility will expand our capabilities in dissecting the control mechanisms of the mammalian circadian system.

Proposed Course:

During the following year we intend to replicate our studies with clorgyline in order in increase the total experimental population. In addition our studies will be extended in order to determine a more complete mechanism of clorgyline's effects. To discriminate pacemaker from slave oscillator effects we plan to derive a light phase response curve in chronic clorgyline treated hamsters. We will also test the phase shifting effects of various neurotransmitters on the circadian pacemaker. We plan to identify the site of clorgyline's action by local microinfusion of the chemical to pacemaker and its input pathways. Third we will determine the specificity of the MAOI effect by studying the effect of reversible MAOI's on the circadian system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00274-11 LCS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methods of Ionization in Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS
Department of Pharmacology, George Washington University, Washington, D.C.

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	.2	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new Fourier transform mass spectrometer has been acquired with laser desorption and capillary gas chromatographic inlets. Initial success in the direct analysis of quaternary pyridinium salts of low molecular weight by laser desorption has been achieved. A mass resolution of 533,000 at m/z 185 was recorded for 1-methyl-4- (4-aminophenyl) pyridinium chloride. Preliminary experiments on the high resolution mass detection of compounds emerging from the gas chromatograph have been initiated.

The energy distribution of organic ions sputtered from liquid matrices has been studied with an experimental surface ionization mass spectrometer. Several amino acids in various viscous organic liquids have been used for this study to quantify and understand the parameters which influence the signal intensity variations which obtain. The systematic determination of energy profiles may be used to predict the appropriate liquid matrix and additives to be used for best analytical advantage.

A microwave discharge interface has been modified to accomodate solid samples as well as gas chromatographic effluents. Sulfur containing peptides and proteins were studied to demonstrate the potential of this apparatus to quantitatively determine the sulfur content in each sample.

PHS 6040 (Rev 1/84) GPO 904-917

Other Professional Personnel Engaged on Project:

Leonid Kelner	Visiting Scientist	BEIB, DRS
Fred P. Abramson	Guest Worker	Professor, Department of Pharmacology, G.W. Univ. Wash. D.C.

Project Description

Objective:

Improvement of methods in analytical biochemistry requires improvement in instrumentation and an understanding of the operative factors in instrumental performance. Surface ionization techniques offer considerable advantages over gas phase methods, particularly in the ability to analyze polar, non-volatile, complex organic molecules without extensive chemical pretreatment.

Methods Employed:

Mass spectrometric instrumentation is designed and built or purchased as required to meet the above objectives.

Major Findings:

A new Fourier transform mass spectrometer (FTMS-2000), the first of its kind has been installed and has passed initial performance tests. This instrument efficiently traps ions formed in a superconducting magnetic field, transmits them to an analytical cell, and determines accurately and precisely their mass-to-charge ratio. The principles are considerably different from all previously employed mass spectrometers in this institution, necessitating new approaches to the design of analytical determinations. A capillary gas chromatographic interface is being tested with former assays (i.e., MPTP measurement, catecholeamine assays) to determine if high resolution mass analysis affords any significant assay improvement. An alternative mode of ionization, laser desorption, is being tested for sensitivity and suitability for direct analysis of non-volatile compounds. For example, 6-hydroxymelatonin sulfate, presently measured by a complex gc-ms procedure, may be directly analyzed by laser desorption FTMS. To date, the instrument has been tested only with standards and synthetic products. A newly synthesized compound, 1-methyl-4-(4-aminophenyl)-pyridinum chloride was characterized by laser desorption FTMS and the molecular weight was determined with a mass accuracy of 3.1 ppm with a resolution of 533,000, demonstrating the instrument's high resolution and mass accuracy capabilities.

The collaborative project with BEIB on surface ionization mass spectrometry has concentrated on quantifying energy distributions of ions evolved from the surfaces of liquid matrices. A complete summary of this work is in Project No. Z01 RS 100 73-05 BEI.

The microwave powered chemical reaction interface was modified for the introduction of solid samples. Samples are placed on a solid quartz rod which is introduced into the reaction zone through a vacuum lock. The energy of the plasma in the reaction zone is sufficient to convert complex organic molecules into single polyatomic species. By using carbon dioxide as a reactant gas, sufficient O_2 is generated to combust samples without introducing a large oxygen load into the mass spectrometer. Thus, the presence of sulfur can be measured by the intensity of m/z 64 from SO_2.

The total amount of organic carbon is measured in a separate experiment using nitrogen as the reagent gas, and measuring the intensity of m/z 27 from HCN. Samples of polymethionine from 20 ng to 9 ug were used to determine the linearity of response and sensitivity of this technique. A range of peptides and proteins containing sulfur were analyzed, and ratios of SO_2 to HCN determined from 1 ug samples. There data demonstrate that the microwave reaction interface is capable of providing quantitative information about a wide variety of compounds.

Significance to Biomedical Research:

Structure elucidation of unknown compounds in complex mixtures, or the specific detection and quantification of known compounds remain important areas of biomedical research. Polar, non-volatile compounds are a particular problem amenable to newer ionization methods. Progress on projects which require these analytical methodologies can be substantially speeded, especially in areas such as drug metabolism and the determination of unknown compounds with biological activity.

Proposed Course:

Most effort will be directed toward the assessment of properties of the new Fourier transform mass spectometer. Experiments to determine the most useful and sensitive means to analyze neuropeptides by laser desorption will receive highest priority. The effects of various means of sample deposition, surface material, concentration, matrix, etc. will be systematically studied. The successful analysis of preformed ions, such as the pyridinium ion, suggest the attachment of ionized functional groups to simple molecules to promote their detection. Catecholamines will be quaternarized with iodomethane and their detection limits determined in mixtures.

Publications:

See Project No. Z01 RS 10073-04-BEI

Abramson, F.P. and Markey, S.P.: A method for the analysis of sulfur in microgram quantities of biological macromolecules. Proc. 33rd Annual Conf. on Mass Spectrometry and Allied Topics, San Diego, in press, 1985.

Markey, S.P. : Recent developments in mass spectometry. J. Clin. Pharm., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00276-06 LCS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Melatonin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (if any)

Section on Neuroendocrinology, LDN, NICHD
Section on Clinical Pharmacology, LCS, NIMH
Section on Neurocytology, LNNS, NINCDS
Section on Brain Aging and Dementia, LN, NIA
Department of Pediatrics, USUHS
Clin. Pychobiol. Branch, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.4	.2	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major urinary metabolite of the pineal hormone melatonin, 6-hydroxymelatonin is being quantified by gas chromatography-negative chemical ionization mass spectrometry. Urinary excretion rates of this metabolite are being used to determine the possible role of the pineal gland in human reproductive biology-i.e., its function during pubertal development and throughout the menstrual cycle. A correlative study of plasma melatonin levels, as measured by radioimmunoassay, and 6-hydroxymelatonin in a group of 22 women has been completed. It validates the comparison of plasma levels of the hormone or urinary levels of its metabolite to assess pineal gland production of melatonin in humans. A four year longitudinal collection of urines for melatonin metabolite excretion by young girls through pubertal development has been completed, and the samples are being analyzed. Collaborative studies on the transplantation of neonatal rat pineals into pinealectomized rats have continued, as have collaborative clinical studies on effects of tricyclic antidepressants on the pineal.

PHS 6040 (Rev. 1/84) GPO 904-917

Other Professional Personnel Engaged on Project:

Lawrence Tamarkin	Biochemist	CPB, NIMH
Robert Golden	Medical Staff Fellow	SCP, NIHM
Merrily A. Poth	Asst. Prof. Pediatrics	USUHS
David C. Klein	Chief, Section on Neuroendocrinology	LDN, NICHD
Milton W. Brightman	Chief, Neurocytology Section	LNNS, NINCDS

Project Description

Objectives:

The role of the pineal gland in human physiology remains undefined. Previously, we developed an assay for the major urinary metabolite of melatonin, the conjugated form of 6-hydroxymelatonin. The urinary assay of this metabolite has been shown to be selective and specific for the pineal hormone, varying diurnally in normal primates, and missing from pinealectomized monkeys or neuronally deficient humans. We have applied the urinary assay to determine whether pineal function is related to pubertal development. This requires daily monitoring of pineal function which can be achieved best by an integrative measure of the urinary metabolite excretion. We have validated this approach with a collaborative study on plasma melatonin levels as correlated to 6-hydroxymelatonin excretion. The assay of 6-hydroxymelatonin also provides a measure of pineal function useful for pharmacological investigations and various animal studies. Implantation of the pineal gland into pinealectomized rats affords an opportunity to re-establish a circadian oscillating gland in the brain and refine techniques of tissue transplantation which might be applied to other neural tissue transplants. Collaborative clinical studies on the effects of tricyclic antidepressants on humans are continuing.

Methods Employed:

Urines have been collected from human voluteers in 8-hour aliquots to permit some measure of diurnal variation. Conjugated 6-hydroxymelatonin was quantified using gas chromatography-negative chemical ionization mass spectrometry.

Major Findings:

The pattern of daily excretion of 6-hydroxymelatonin in young girls studied over a 4-year period did not show a significant change with pubertal development. The interim findings suggested that the pineal gland produces a constant, but individually distinct amount of melatonin in pre-menarchal girls. These urines are now being reanalyzed as sample sets for each girl, rather than as group sets for each collection time. Subtle differences, if present, can be detected, and changes in the excretion pattern as compared to the total amplitude, determined.

A significant correlation (0.76) was found between nighttime peak plasma melatonin levels and the 24-hr urinary excretion totals for conjugated 6-hydroxymelatonin for a group of 22 women. This group included 18 healthy female volunteers ranging in age from 18-68 yr (mean 44 $\pm$ 10.4). All subjects were admitted to the Clinical Center as subjects in a study on estrogen receptors and melatonin production. Blood samples were drawn every 3 hr for 24 hr, and urine samples pooled for three 8-hr time periods. The best correlation between radioimmunoassayable plasma melatonin and the urinary metabolite levels was found between the nighttime peak plasma levels

and total urinary excretion values. This study demonstrates that subjects with high plasma melatonin levels excrete relatively large amounts of metabolite and that those with low or undetectable plasma melatonin excrete low or unmeasureable amounts of metabolite. The correlation precludes major differences in metabolic turnover as effecting either measure, demonstrating that both measurements reflect pineal production differences. This study validates the comparison of plasma levels of the hormone or urinary levels of its metabolite to assess pineal gland production in humans.

Assays of urines from pinealectomized and pineal transplated rats have thus far not shown complete regeneration of pineal gland function, although there is a constant but reduced production of 6-hydroxymelatonin (day and night) in transplanted animals.

Melatonin production may provide an index of beta-noradrenergic function as it is affected by various pharmacological agents. For one trial group treated with desipramine, 6-hydroxymelatonin excretion increased by 64%, confirming the hypothesis that an increase in noradrenergic function is involved in the antidepressant action.

Significance to Biomedical Research:

Studies on the normal physiological role of melatonin in human biochemistry are lacking. This project is intended to gather baseline data and define the possible role of melatonin in several of its most frequently cited functions. Postulates regarding altered melatonin production as a consequence of altered circadian function and their relation to mental health require these data.

Proposed Course:

Reanalysis of the longitudiually collected urine from young girls throughout various stages of puberty is in progress. Alternative methods for the analysis of 6-hydroxymelatonin will be evaluated. Collaborative studies on the use of 6-hydroxymelatonin as an index of beta-adrenergic function will be continued.

Publications:

Higa S., and Markey, S.P. : Indentificatin and quantification 5-methoxyindole-3-acetic acid in human urine. Anal. Biochem. 144 : 86-93, 1985.

Markey, S.P., Higa, S., Shih, M., Danforth, D.N., and Tamarkin, L. : The correlation between human plasma melatonin levels and urinary 6-hydroxymelatonin excretion. Clinica. Chimica. Acta, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00277-06 LCS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Stable Isotope Labeled Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Stable and some radioisotope labeled compounds have been synthesized to support other laboratory projects. Structural analogues of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine were prepared (see Z01 MH 00279-03 LCS). The synthesis of $^{13}C_6$-(phenyl)-labeled norepinephrine from guaiacol-(phenyl-$^{13}C_6$) has been initiated.

PHS 6040 (Rev. 1/84) GPO 904-917

Other Professional Personnel Engaged on Project:

Adrian Weisz	Visiting Fellow	SAB, LCS

Project Description

Objectives:

The synthesis of labeled compounds is an integral support function to investigations of metabolism and distribution of endogenous and xenobiotic compounds. Mass spectrometric studies on the metabolism and kinetics of the neurotransmitter norepinephrine require the use of a stable isotopomer. A phenyl-$^{13}C_6$-labelled norepinephrine is the current synthetic objective.

Methods Employed:

Conventional methods of chemical synthesis employing isotopes have been utilized.

Major Findings:

A synthetic route beginning with commercially available $^{13}C_6$-guaiacol has been tested with unlabeled materials and is being applied to the labeled precursors. Guaiacol is converted quantitatively to catechol, condensed with the chloride of glycine-phthalimide, which is then deblocked by hydrolysis, and catalytically reduced to norepinephrine.

Significance to Biomedical Research:

The availability of labeled compounds is frequently the limiting step in metabolism projects. A program in analytical biochemistry requires continuing synthetic efforts to prepare stable and radioisotope analogues for the timely and efficient solution to metabolism projects.

Proposed Course:

The completion of the $^{13}C_6$-norepinephrine synthesis, and resolution of the resulting stereoisomers will receive greatest priority. Labeled and structural analogs of MPTP will be prepared for metabolic studies.

Publications:

None (see Z01 MH 00279-03)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00279-03 LCS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology of Neurotoxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry

COOPERATING UNITS (if any)

Section on Histopharmacology, LCS, NIMH
Laboratory of Neurophsiology, NIMH Office of the Director, IRP, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.4	2.0	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanism of action of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is being studied in several animal species. In primates, MPTP produces a persistent parkinsonian syndrome. In dogs, MPTP destroys cells in the A-9 cell group in the substantia nigra, and does not kill other cell types. In the rat, no nigral cell death has been induced, but in mice long-lasting impairment of nigral neuronal function has been documented. The metabolic fate of MPTP in the monkey and rodent is consistent with species difference in metabolism being important for susceptability. MPTP is converted to the pyridinium ion MPP^+ in all species, but trapped intraneuronally for long times in the monkey. Blockade of the oxidation of MPTP to MPP^+ with monoamine oxidase type B inhibitors prevents the toxicity of MPTP in mice and dogs. The oxidation of MPTP to MPP^+ has been studied *in vitro* in primate tissue.

The role of oxidative stress produced by trapped MPP^+ has been studied in the rodent. MPP^+ administered peripherally causes lung damage to the rat, similar to that produced by its structural analog paraquat. Furthermore, it caused an increase in plasma glutathione disulfide 60-90 minutes after administration. Selenium deficient mice which are especially susceptible to oxidative stress exhibited lowered LD_{50} levels toward MPP^+. This oxidative stress mechanism may play a role in the CNS toxicity of MPTP.

A sensitive and specific gas chromatographic-mass spectometric quantitative assay for MPTP and MPP^+ has been developed and applied to the studies of its toxic mechanism. The synthesis of analogs of MPTP and MPP+, and the synthesis of antigens for antibody preparation to MPTP and MPP^+ is in progress.

Other Professional Personnel Engaged on Project:

Chuang Chiueh	Special Expert	OD, IRP, NINCDS
Jan Johannessen	Staff Fellow	SAB, LCS, NIMH
Richard S. Burns	Senior Staff Neurologist	OD, IRP, NINCDS
Irwin J. Kopin	Director	IRP, NINCDS
David Jacobowitz	Chief	SH, LCS, NIMH
Miles A. Herkenham	Research Psychologist	LNP, NIMH

Project Description

Objectives:

The neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in man and monkey has been described. Relatively small amounts (.3-.5 mg/kg) administered peripherally over five days produce a parkinsonian sydrome in primates which neurochemically and histopathologically resembles idiopathic Parkinson's disease in humans. An animal model of this disorder has long been sought, but other lesioning techniques have failed to produce a persistent condition with all of the neurochemical and locomotor effects observed in the human disorder and MPTP exposure. The mechanism of this neurotoxicity is particularly interesting in that larger doses of MPTP given chronically to rats, cats, or guinea pigs fail to produce any observable locomotor deficit, although measurable dimunition of dopamine in the nigro-striatal system is apparent in the rat and the guinea pig. The objectives of this project are to unravel the mechanism of MPTP neurotoxicity in the primate, particularly with regard to its specific effects on the caudate nucleus and putamen, and lack of effect on the nucleus accumbens.

Methods Employed:

MPTP toxicity is being studied by qualitative and quantitative observation of animal behavior and locomotion; neurochemical determination of catecholamines and their metabolites in specific brain regions by high pressure liquid chromatography (HPLC) with electrochemical detection; determination of the pattern of MPTP distribution, metabolism, and excretion, using radio and stable isotope labelled MPTP (^{3}H, ^{14}C, ^{2}H) in several animal species; identification of metabolites unique to the primate and synthesis and pharmacological testing of candidate metabolites. These methods rely upon high specific activity MPTP prepared in this study to characterize by HPLC labeled metabolites extracted from physiological fluids or tissues. Autoradiography is being employed to study tissue localization. The structures of isolated metabolites are being determined by mass spectrometry, and will be synthesized and tested *in vivo* to measure their neurotoxicity. Receptor binding studies of MPTP and its metabolites with respect to known or other specific receptors is being pursued.

Major Findings:

MPTP neurotoxicity is secondary to its metabolism. This has been shown by studies which first characterized the principal brain metabolite of MPTP in primates as 1-methyl-4-phenylpyridinium ion (MPP^{+}), and secondly showed that blockade of this metabolic transformation eliminated toxic damage in the mouse brain. Rhesus monkeys were injected intravenously with $^{3}H_{2}$-MPTP, ^{14}C-MPTP, and unlabelled MPTP, and killed between 1 and 20 days after injection. Their brains were removed and studied by autoradiography and dissection procedures. The radioactivity present in all brains was characterized as being predominantly (greater than 90%) due to MPP^{+} both by

liquid chromatographic procedures and by mass spectometric methods. Experiments where $^{3}H_2$-MPTP and ^{14}C-MPTP were given at staggered times failed to indicate any regional differentiation in label concentration, precluding the retrograde transmission of MPTP from terminal areas to cell bodies. In the mouse, MPTP was found to be rapidly converted in brain to MPP^+, but unlike in the monkey, to be rapidly eliminated from the brain either by subsequent metabolism or another mechanism. Pretreatment of mice with the monoamine oxidase inhibitor pargyline blocked the enzymatic conversion of MPTP to MPP^+ as measured mass spectrometrically. Furthermore, it led to higher levels of MPTP being present in the mouse brain. The effect of pargyline pretreatment was to block the neurotoxic effects of MPTP as determined by dopamine levels in the mouse neostriatum. MPTP alone produces an 80-90% depletion in dopamine levels in the caudate region, and this can be blocked with pargyline.

Other studies have shown that neither the presence of MPTP nor conversion to its metabolite MPP^+ is sufficient to produce neurotoxicity to a cell. The in vitro oxidation of MPTP to MPP^+ was demonstrated to be a robust process for many brain regions from the rhesus monkey. The selective lesion of nigrostriatal neurons was demonstrated by immunocytochemical staining of the mid-brain with a tyrosine hydroxylase antiserum. Cell bodies in the dopaminergic A9 region, but not in the A10 ventrotegmental area appeared to be susceptible. Despite greater than 80% decrease in A9 nigral cell bodies, dopamine content decreased by only 50%. Sprouting of the surviving A9 neurons was observed histologically and neurochemically in the area above the substantia nigra. Thus it may be that the regenerative process is more active in the mouse than in other species, rather then there being a difference in the basic neurotoxic mechanism between species.

The metabolite MPP^+ is itself toxic when present in sufficient concentration. Direct injection into neural tissue produces a caustic and non-specific lesion. However, MPP^+ has been studied in the periphery in the rodent. In analogy to paraquat, a structural analog, MPP^+ may produce oxidative stress by forming free radicals through a one-electron reduction. If a futile cycle is established between MPP^+ and its free radical, with the free radical reducing oxygen to superoxide ion and eventually producing hydrogen peroxide, then this could be detected by an increase in the levels of glutathione disulfide. Near lethal doses of MPP^+ administered to rats were found to produce 4-fold increases in rat plasma glutathione disulfide after 60-90 minutes. Examination of the pathology produced by MPP^+ established that it is a very selective pulmonary toxin. Thus, oxidative stress is one mechanism by which MPP^+ may play a role in the toxicity of MPTP in the brain.

Significance to Biomedical Research:

The MPTP lesioned primate and dog are the best animal models of idiopathic Parkinson's disease which have been described. The mechanism of action of MPTP may be relevant to the cause of Parkinson's disease. The etiology of certain forms of parkinsonism may be due to MPTP-like environmental factors.

Proposed Course:

Structure-activity probes of MPTP analogs will be performed in mice and dogs to determine if the mechanism of toxicity is parallel in both species and to enumerate the key structural features of MPTP with regard to it's toxicity. An analog with neurotoxic properties which can be fixed in tissues will be tested. Antibodies to MPTP and MPP^+ will be raised and tested for specificity. The metabolsim of ^{14}C-ring labeled MPTP will be studied in several species.

Publications:

Chiueh, C.C., Burns, R.S., Markey, S.P., Johannessen, J.N. Jacobowitz, D., Ebert, M.H., and Kopin, I.J. : Neurochemical and behavioral effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rat, guinea pig, and monkey. Psychopharmacol. Bull. 20: 548-553, 1984.

Johannessen, J.N. and Markey, S.P. : Assessment of the opiate properties of two constituents of a toxic illicit drug mixture. Drug Alcohol Depend., 13: 367-74, 1984.

Markey, S.P., Johannessen, J.N., Chiueh, C.C., Burns, R.S., and Herkenham, M.A.: Intraneuronal generation of a pyridinum metabolite may cause drug-induced parkinsonism. Nature 311: 464-467, 1985.

Chiueh, C.C., Burns, R.S., Markey, S.P., Jacobowitz, D.M. and Kopin,I.J.: Primate model of parkinsonism: selective lesion of nigrostriatal neurons by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine produces an extrapyramidal syndrome in rhesus monkeys. Life Sci. 36: 213-218. 1985.

Johannessen, J.N., Kelner, L., Hanselman, D., Shih, M. and Markey, S.P.: In vitro oxidation of MPTP by primate neural tissue: a potential model of MPTP neurotoxicity. Neurochem. Int. 7: 169-176, 1985.

Johannessen, J.N., Chiueh, C.C., Burns, R.S. and Markey, S.P. : Differ -ences in the metabolism of MPTP in the rodent and primate parallel differ -ences in sensitivity to its neurotoxic effects. Life Sci. 36: 129-224. 1985.

Shih, M. and Markey, S.P. : Combined quantitative determination of MPTP and MPP^+ in mouse brain tissue by gas chromatography-mass spectrometry. MPTP-A Parkinsonian Syndrome Producing Neurotoxin (Eds. S.P. Markey, N. Castagnoli, I.J. Kopin, A.J. Trevor), in press.

Adams, J.D., Johannessen, J.N., Schuller, H., Bacon J.P. and Markey,S.P.: The role of oxidative stress in the toxicity of MPP^+ and MPTP in the rodent. MPTP-A Parkinsonian Syndrome Producing Neurotoxin (Eds. S.P. Markey N. Castagnoli, I.J. Kopin, A.J. Trevor), in press.

Johannessen, J.N., Chiueh, C.C., Herkenham, M.A. and Markey, S.P. : Relationship of the in vivo metabolism of MPTP to toxicity. MPTP-A Parkinsonian Syndrome Producing Neurotoxin (Eds. S.P. Markey, N. Castagnoli, I.J. Kopin, A.J. Trevor), in Press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02241-01 LCS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Positron Emission Tomographic Imaging of Neurotransmitters and their Turnover

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

C. C. Chiueh Special Expert LCS NINCDS

COOPERATING UNITS *(if any)*

LCM, NIMH; LC, NIADDK; NM, CC, NIH; OB, FDA; OD, NINCDS and Dept. of Nuclear Medicine, McMaster University Medical Centre, Hamilton, Ontario, Canada.

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section of Analytical Biochemistry

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The present results indicate that the addition of a fluorine moiety to the C-6 position of L-dopa and dopamine decreases their vulnerability to catabolism by catechol-O-methyl-transferase. This, in turn, drastically decreases the nonspecific background in positron emission tomography (PET) activity. The major metabolite of 6-F-dopa in the caudate nucleus is 6-F-dopamine, rather than O-methyl-6-F-dopa. The turnover rate of 6-fluoro-catecholamine resembles that of ^{3}H-catecholamine. This neuro-chemical evidence is the basis for the use of the presynaptic ligand fluorine-18 labeled 6-F-dopa in a PET procedure for imaging brain dopamine. By using the high resolution McMaster PET scanner, brain damage to striatal dopamine of MPTP-induced parkinsonian monkeys was visualized and quantified by using fluorine-18 labeled 6-F-dopa. The results indicate that the accumulation of fluorine-18 PET activity due to 6-F-dopamine is absent in a severe case of parkinsonism. This preclinical trial has demonstrated that the degree of brain damage in living parkinsonian monkeys appears to be quantifiable by this PET procedure. Thus, 6-F-dopa has fulfilled the criteria of an ideal presynaptic ligand for PET imaging of brain dopamine. This PET imaging procedure may prove to be a diagnostic tool for the detection and quantification of subclinical cases of Parkinson's disease after passing a preclinical toxicological evaluation. It has further been proposed to modify this PET procedure to measure the turnover rate of dopamine in the mesocortical and mesolimbic systems and to evaluate the dopaminergic mechanism of clinical neuropyschiatric disorders.

Other Professional Personnel Engaged on the Research Project:

R. M. Cohen	Section Chief	LCM	NIMH
K. L. Kirk	Sr. Chemist	LC	NIADDK
S. M. Larson	Director	NM CC	NIH
J. L. Sun	Pharmacologist	OB	FDA
I. J. Kopin	Director	IRP OD	NINCDS
G. Firnau	Chemist	Dept. of Nuclear Medicine	
C. Nahmias	Computer Engineer	McMaster University	
E. S. Garnett	Director	Medical Centre	
		Hamilton, Ontario, Canada	

Project Description

Objectives:

The goals of this project are to develop an ideal presynaptic positron emitting (either carbon-11 or fluorine-18 labeled) ligand for imaging brain dopaminergic systems and for visualizing their functional turnover rate in vivo. Such a noninvasive positron emission tomographic (PET) procedure may prove to be useful in clinics for determining brain damage in Parkinson's disease and for evaluation of the dopaminergic mechanism in neuropsychiatric disorders.

Methods Employed:

1. Synthesis of Tritium and Fluorine-18 Labeled 6-F-dopa (6-fluoro-dihydroxyphenylalanine):

Radioactive labeled compounds are synthesized and provided by Dr. Gunter Firnau (1984) and Dr. Kenneth Kirk (1984) using their previously described procedures.

2. Animals:

Adult rhesus monkeys (Macaca mulatta) of both sexes (5-8 kg) and O-M female rats were used. The monkeys were housed individually in primate quarters on a 12-hr light/dark cycle. Purina monkey chow, water, juice, and fresh fruit were given ad lib. In severely affected parkinsonian monkeys, liquid food was force-fed through a nasogastric tube and required L-dopa therapy (Sinemet 100/10, q.i.d.) was given. Parkinsonism is induced in monkeys after an intravenous administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in doses of 0.5 to 2.5 mg/kg to the animals. Rats were housed in groups of 5-6 animals per cage in rodent quarters on 12-hr light cycle (0600-1800 hr). Food and water were given ad lib. Rats (200g) were pretreated with a peripheral decarboxylase inhibitor (MK-486, 75 mg/kg, i.p., 60 min). Tritium labeled 6-F-dopa (7.8-[^{3}H], sp. act. 2.5 mCi/mmole) was dissolved in saline (1 uCi/g) and administered through a venous catheter placed in the tail vein. Rats were sacrificed at various times after the treatment.

3. Neurochemical Procedures:

The metabolites of 6-F-dopa, i.e. 6-F-dopamine, 6-F-dihydroxy- phenylacetic acid, 6-F-homovanillic acid and O-methylated 6-F-dopa were separated and quantified by a high performance liquid chromatographic procedure.

4. Positron Emission Tomographic Imaging of Brain Dopamine:

Following an intravenous injection of 1.5 to 2 mCi purified 6-^{18}F-dopa (sp. act. 75 mCi/mmol), pentobarbital anesthetized monkeys were examined in the McMaster PET scanner. The total radioactivity attributed to 6-F-dopa and its metabolites in a 10 mm horizontal brain slice along the orbitomeatal plane was measured for 2 to 3 hours. At various intervals, blood samples were drawn and assayed for 3-methyl- 6-F-dopa, which was found to disappear within about 40 min. Each animal was sacrificed at the end of the experiment. Disc shape punches of tissue from the caudate nucleus and the putamen were assayed for dopamine and its biosynthetic enzymes.

Major Findings:

Our initial study indicated that 6-fluoro-catecholamine, by fulfilling the criteria for a false adrenergic transmitter, may be useful in PET scanning of the adrenergic nervous system in the brain or in the peripheral sympathetic nervous system.

In the present study, we synthesized tritium-labeled 7,8-[^{3}H]-6-F-dopa (2.6 Ci/mmole) and investigated the metabolism of this compound in the plasma and the caudate nucleus. The present results provide a neurochemical basis for the use of 6-F-dopa in brain dopamine PET imaging procedure.

In the caudate nucleus, tritium counts associated with 6-F-dopa, 6-F-dopamine, 6-F-dopac, 3-methyl-6-F-dopa, and 6-F-homovanillic acid were detectable. The major peaks were 6-F-dopamine (295 cpm/mg, peaked 30 min) and 6-F-dopac (97 cpm/mg, peaked 15 min). Striatal levels of other metabolites of ^{3}H-6-F-dopa were less than 100 cpm/mg throughout the experimental period. There was no sign of accumulation of ^{3}H-3-methyl-6-F-dopa in the brain. The total counts consisted mainly of 6-F-dopamine (about 60%). It reached 500 cpm/mg 30 min after ^{3}H-6-F-dopa and decreased with a half-life of 70 min. The estimated rate of accumulation of 6-F-dopamine in the caudate nucleus was about 75 fmole/mg/15 min while its rate of disappearance was approximately 25 fmole/mg/15 min.

The MPTP-induced brain damage to the basal ganglia in parkinsonian monkeys was visualized by the PET procedure using fluorine-18 labeled 6-F-dopa. In controls, the striatal ^{18}F-PET activity was maximum at 15 min and declined with a half-life of 5 hours. The turnover rate of striatal ^{18}F-activity was diminished 3 days after MPTP administration. Two to three weeks later, the accumulation of 6-^{18}F-dopa and its major metabolite, 6-^{18}F-dopamine, was diminished in the basal ganglia of the mildly affected monkey and absent in the severely affected animal. The striatal ^{18}F-dopamine activity calculated by PET *in vivo* correlates with the content of endogenous dopamine measured postmortum in each animal.

Significance to Biomedical Research:

The present results suggest that 6-^{18}F-dopa has fulfilled the criteria for presynaptic PET ligand of brain dopamine. This PET brain ^{18}F-dopamine imaging may be useful in providing an index of dopamine depletion in patients with Parkinson's disease and allied disorders.

The degree of brain damage in patients suffering from Parkinson's disease depends upon clinical assessments and confirmation by postmortem examination. It may be particularly useful to apply the knowledge obtained from the present research project of PET imaging of brain dopamine of the primate model of parkinsonism in classifying patients at the earliest sign of the disease. The possibility that the progression of clinical parkinsonism might be delayed and/or prevented has been offered hope by the recent advance in prevention of MPTP's neurotoxicity by pargyline and deprenyl. By using the monkey model of parkinsonism, we could also develop and test new medications (i.e. deprenyl, oxygen free radical inhibitors, etc.) for the prevention of clinical progression of parkinsonism in order to prolong the patient's life span and improve their quality of life.

Proposed Course:

1. Preclinical Studies:

In collaboration with Dr. S. Larson, the Director of the Department of Nuclear Medicine, this PET imaging procedure will be set up at the NIH Clinical Center. This procedure includes the generation of fluorine-18 by a cyclotron, the fluorination of L-dopa, and the isolation of fluorine-18 labeled 6F-dopa. Dr. Firnau, of the McMaster Unversity Medical Centre, has agreed to serve as a consultant in the preparation of this presynaptic dopamine ligand for this project. Before we apply the brain imaging procedure to human subjects, additional toxicological and pharmacological evaluations will be conducted in experimental animals in order to meet the safety guidelines stipulated by the Food and Drug Administration.

The present findings also indicate that this procedure could be used to measure the functional turnover rate of brain dopamine in vivo. More efforts will be focused on the procedure for measuring the in vivo turnover rate of dopamine in the mesolimbic and mesocortical systems, which control functions such as mood, emotion, and memory, etc. The use of this procedure to explore and evaluate the dopaminergic mechanism in neuropsychiatric disorders will be initiated.

2. Clinical Studies:

After passing the preclinical toxicological evaluations, this presynaptic dopamine ligand will be administered to normal volunteers, parkinsonian patients, bipolar manic-depressive patients, and patients suffering from other disorders which involve brain dopamine. These clinical projects will be conducted following established NIH guidelines and with the collaboration of different clinical branches of NIH and NIMH.

Publications:

Chiueh, C.C., Firnau, G., Burns, R.S., Nahmias, C., Chirakal, R., Kopin, I.J., and Garnett, E.S.: Determination and visualization of damage to striatal dopaminergic terminals in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism by fluorine-18 labeled 6F-L-DOPA and positron emission tomography. In Yahr, M.D. and Duvoisin, R.C. (Ed.): Parkinson's Disease: Advances in Neurological Series. New York, Raven Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00351-11-LCS

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Pharmacology of the Central Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David C. Jimerson, Chief, Section on Biomedical Psychiatry

COOPERATING UNITS *(if any)*

SAB, LCS, NIMH; ETB, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Biomedical Psychiatry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to develop techniques for studying central nervous system monoamine metabolism in man, and to assess the value of peripheral neurotransmitter measures as indices of central function. Clinical studies evaluating alterations of norepinephrine metabolites in plasma were continued. Differential relationships between norepinephrine activity and cortisol levels were demonstrated in healthy volunteers and bulimic patients, in comparison to previous results in depressed patients. A study of a proposed new antidepressant drug rolipram on brain norepinephrine function was performed in laboratory rodents.

Other Professional Personnel Engaged on Project:

S.P. Markey	Chief, Section on Analytical Biochemistry	LCS, NIMH
D. Lozovsky	Visiting Scientist	ETB, NINCDS
H.E. Gwirtsman	Medical Staff Fellow	LCS, NIMH

Project Description:

Objectives:

The purpose of this study is to develop techniques for the comparative assessment of central and peripheral nervous system monoamine metabolism in man. This work includes preliminary studies in laboratory animals and the development and validation of new biochemical assays when necessary. Application of these techniques is assessed in studies of psychiatric patients and control groups.

Methods:

Biochemical methods for assay of endogenous catecholamine metabolites in tissues and body fluids include gas chromatography-mass spectrometry (GCMS) and high pressure liquid chromatography. Radioimmunoassay techniques are used for assay of hormone levels regulated by catecholamine neurotransmitters in vivo. Physiological regulation of neurotransmitter turnover and receptor function is evaluated in laboratory rodents using brain tissue, blood, or urine as necessary.

Major Findings:

In conjunction with clinical studies, interaction between the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system was a topic of focus. In previous studies we demonstrated a positive correlation between plasma levels of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) and plasma cortisol in depressed patients. Patients showing impaired suppression of cortisol following 1.0 mg of dexamethasone (non-suppressors) had higher MHPG levels than suppressors. Over the past year we studied a group of healthy volunteers and found an inverse correlation between HPA axis activity (as reflected in urinary excretion of free cortisol) and plasma MHPG. In a group of medication free, nutritionally stabilized women with bulimia, we found a substantial incidence of non-suppression on dexamethasone testing. In contrast to results in depressed patients, however, plasma MHPG levels were not different for suppressor and non-suppressor patient groups. This finding suggests that the positive correlation between HPA and sympathetic nervous system activation observed in some depressed patients may have syndromal specificity, and needs to be assessed in other patient groups.

Recent studies using laboratory animal models of depression and preliminary open clinical trials have suggested that the cyclic-AMP phosphodiesterase inhibitor rolipram may have antidepressant effects. Proposed mechanisms for the antidepressant effect include increased norepinephrine turnover in brain as well as phosphodiesterase inhibition. These results suggested that rolipram might have in common with other antidepressants the property of down-regulating cortical beta-adrenoceptors with chronic treatment. Our studies in Sprague-Dawley rats failed to demonstrate an effect of acute or chronic rolipram treatment on ^{3}H-dihydro-alprenolol binding in brain. We did find that when combined with desipramine treatment, rolipram accelerated the onset of desipramine-induced down-regulation of cortical beta-adrenoceptors. This observation needs to be tested in other animal models, but does suggest a possible strategy for accelerating antidepressant responses in clinical settings.

Significance to Biomedical Research and the Program of the Institute:

Assessing the rate and pathways of amine metabolite production and clearance in laboratory animals and patients provides information on the rate and location of neurotransmitter turnover. Better understanding of the relationship betwen blood, urine and cerebrospinal fluid metabolite levels may help localize the alteration in catecholamine turnover observed in psychiatric syndromes. These questions extend to possible sites of interaction between neurotransmitter and hormone neuromodulators. Evaluation of the behavioral effects of drugs affecting neurotransmitter activity may help further clarify the role of specific neurotransmitter alterations in major psychiatric syndromes.

Proposed Course:

Future studies will explore new strategies for applying neurotransmitter metabolite levels and their ratios in assessing neurochemical function in psychiatric patients and healthy volunteers. Sites of functional interaction between catecholamines and hormone systems in patient groups will be assessed through neurochemical assys and pharmacological challenge studies. The effects of newer antidepressant and anxiolytic drugs on neurotransmitter function will be assessed in laboratory animals and psychiatric patients. In conjunction with proposed pharmacologic studies assessing animal models of bulimia, we plan to study possible mechanisms of interaction between brain monoamine neurotransmitters and gut-related peptides that are potent modulators of eating behavior.

Publications:

Jimerson, D.C.: Neurotransmitter hypotheses of depression: Research update. Psychiatric Clinics of North America 7: 563-573, 1984.

Ballenger, J.C., Peterson, G.A., Laraia, M., Hucik, A., Lake, C.R., Jimerson, D.C., Cox, D., Trockman, C., Shipe, J., Wilkinson, C.: A study of plasma catecholamines in agoraphobia and the relationship of serum tricyclic levels to treatment response. In Ballenger, J.C. (Ed.): Biology of Agoraphobia. Washington, D.C., American Psychiatric Press, 1984, pp. 27-63.

Jimerson, D.C., Rubinow, D.R., Ballenger, J.C., Post, R.M., and Kopin, I.J.: CSF norepinephrine metabolites in depressed patients: New methodologies. In Usdin, E., Carlsson, A., Dahlstrom, A., and Engel, J. (Eds.): Catecholamines, Part C: Neuropharmacology and Central Nervous System--Therapeutic Aspects. New York, Alan R. Liss, 1984, pp. 123-129.

Jimerson, D.C., and Berrettini, W.B.: Cerebrospinal fluid amine metabolite studies in depression: Research update. In Beckman, H. and Riederer, P. (Eds.): Pathochemical Markers in Major Psychoses. Berlin, Springer Verlag, 1985, pp. 129-143.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00352-09-LCS

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David C. Jimerson, Chief, Section on Biomedical Psychiatry

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Biomedical Psychiatry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been discontinued; clinical studies which are being continued are reported under Project Number Z01 MH 02289-01-LCS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00353-03-LCS

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Biochemical and Pharmacological Studies of Parkinson's Disease and Related Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R. Stanley Burns, M.D., Senior Staff Neurologist, SET, LCS, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Biomedical Psychiatry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been discontinued because the principal investigator moved to NINCDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02289-01-LCS

PERIOD COVERED

October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychobiology of Eating Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David C. Jimerson, Chief, Section on Biomedical Psychiatry

COOPERATING UNITS *(if any)*

SCN, LCS, NIMH; SCP, LCS, NIMH; SCN, BPB, NIMH; CNG, NIMH; LPP, NIMH; SCS, NBS, NIMH; Department of Psychiatry, Vanderbilt University

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Biomedical Psychiatry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical studies over the past year have elucidated patterns of altered neurotransmitter, neuroendocrine, and neuropeptide function in anorexia nervosa and bulimia. Some changes in norepinephrine function in these disorders appear to be independent of weight change per se, in that reduced norepinephrine levels were found in long-term weight recovered anorexic patients and in nutritionally stabilized bulimic subjects. Studies of cortisol and thyroid hormones showed altered regulation, with some changes similar to those reported in depressed patients. Opiate-related peptides in cerebrospinal fluid were reduced in anorexic patients at low weight, but increased with weight restoration. Other studies focused on energy metabolism and brain imaging in anorexic patients. New studies were begun to evaluate the interaction between neurotransmitters and energy metabolism, and to assess the role of serotonin changes in altered appetite and eating patterns observed in these patient groups.

PHS 6040 (Rev. 1/84)

GPO 914-918

Other Professional Personnel Engaged on Project:

W.H. Kaye	Staff Psychiatrist	LPP, NIMH
H.E. Gwirtsman	Medical Staff Fellow	LCS, NIMH
D.T. George	Medical Staff Fellow	LCS, NIMH
T.D. Brewerton	Medical Staff Fellow	LCS, NIMH
E. Obarzanek	Guest Worker	LCS, NIMH
J. Kassett	Clinical Social Worker	LCS, NIMH
W. Berrettini	Staff Psychiatrist	CNG, NIMH
P.W. Gold	Chief, Section on Clinical Neuroendocrinology	BPB, NIMH
E.A. Mueller, III	Medical Staff Fellow	LCS, NIMH
D.L. Murphy	Chief	LCS, NIMH
H. Weingartner	Research Psychologist	LPP, NIMH
M.H. Ebert	Chairman, Dept. of Psychiatry	Vanderbilt Univ.
W.Z. Potter	Chief, Section on Clinical Psychopharmacology	LCS, NIMH
N. Buckholtz	Guest Worker	LCS, NIMH
J. Winterer	Medical Staff Fellow	DEB, NICHD
C. Duncan	Senior Staff Fellow	LPP, NIMH
C. Kellner	Medical Staff Fellow	BPB, NIMH
P. Avgerinos	Visiting Fellow	BPB, NIMH
E. Gershon	Chief	CNG, NIMH
D. Pickar	Chief, Section on Clinical Studies	NSB, NIMH

Project Description

Objectives:

The purpose of this project is to study the psychobiology of major eating disorders from the standpoint of neurotransmitter metabolism, neuroendocrine and neuropeptide function, cognitive and psychological function, and pharmacologic treatment. The major focus of this project is presently directed toward the syndromes of anorexia nervosa and bulimia.

Methods:

1. Behavioral and psychological assessment: Baseline evaluation of patients and control subjects entails clinical and structured diagnostic interviews; subjective and objective mood, attitude and behavioral ratings; and psychometric approaches. Baseline measures and treatment-related alterations are assessed by research psychiatrists, social worker, psychologist, clinical nutritionist and nursing staff.

2. Neurobiologic assessment: Studies of presynaptic neurotransmitter activity include measurement of the biogenic amines and their major metabolites in blood, urine and cerebrospinal fluid. These

studies focus on norepinephrine and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), dopamine and homovanillic acid (HVA), and serotonin and 5-hydroxyindole acetic acid (5-HIAA). Neurotransmitter receptor activity is assessed through challenge studies using selective pharmacologic probes, with measurement of physiologic, metabolic, neuroendocrine and behavioral responses. Receptor sensitivity is measured in vitro using cellular elements from blood.

Methods used in neuroendocrine protocols are established techniques of measuring baseline hormone levels in blood (cortisol, corticotropin (ACTH), prolactin, growth hormone, triiodothyronine, and thyroid stimulating hormone) or urine (free cortisol), as well as responses to hypothalamic releasing factors, other pharmacologic probes, and physiologic challenges. These techniques test the integrity of feedback regulation in specific endocrine systems, as well as the sensitivity of neurohormonal and neurotransmitter receptors. Activity of neuropeptide systems in the central nervous system is evaluated by measurement of cerebrospinal fluid peptide concentrations in a baseline state and during treatment.

Nutritional and metabolic state is evaluated using standard biochemical measures in blood and urine, anthropometry, potassium-40 scanning and (in the coming year) indirect calorimetry. Cerebral electrophysiologic responses are measured in collaboration with investigators in other branches. Cerebral anatomic and functional imaging studies utilize computed tomography, positron emission tomography, and nuclear magnetic resonance techniques.

Major Findings:

From a clinical standpoint the syndromes of anorexia nervosa and bulimia have a number of symptoms in common, including preoccupation with eating patterns, with body image and with body weight, and episodes of depression. Although the studies have been reported separately below for the two patient groups, an important perspective in our analysis of the studies includes comparison of results for anorexic, bulimic and control subjects -- as well as results for depressed and anxiety disorder patients studied with similar paradigms by other NIMH research groups.

A. Anorexia Nervosa

Neurotransmitter turnover and receptor sensitivity. Previous studies by our group and others have demonstrated that norepinephrine turnover is altered in patients with anorexia, as reflected by low levels of norepinephrine or its metabolites in blood, urine and cerebrospinal fluid. Indirect evidence suggests that norepinephrine alterations may play a role in depressive symptoms, neuroendocrine dysfunction and altered energy metabolism observed in anorexia nervosa. Because of evidence that alteration in norepinephrine turnover may result from weight change per se, we have studied neurotransmitter

function in a group of women who had maintained a stable, relatively normal body weight for at least 6 months following a low weight anorexia episode. In these women, levels of norepinephrine and MHPG in plasma and cerebrospinal fluid were significantly decreased in comparison to control values. Norepinephrine levels were not different for women with good or bad prognostic signs (e.g., return of menstrual cycles). Further studies are needed to assess whether these results reflect a role for altered norepinephrine turnover as a premorbid vulnerability factor in anorexia, or possibly a physiological adaptation that makes it easier for these women to sustain their recovered weight.

To obtain an assessment of overall output of norepinephrine systems in anorexia, we have pursued strategies to measure post-synaptic receptor function in conjunction with indices of pre-synaptic activity (neurotransmitter and metabolite levels, as noted above). Completion of the infusion study with the $alpha_2$-agonist clonidine has demonstrated super-sensitive growth homone response in newly admitted severely cachectic patients. Nutritional rehabilitation and weight restoration was associated with marked blunting of both growth hormone and MHPG responses (reflecting, respectively, post-synaptic and pre-synaptic receptor sensitivity). Inferential evidence from animal studies and clinical studies in depressed patients suggests that this down-regulation of $alpha_2$-adrenoceptors may be related to depressive symptoms and further reduction of appetite observed in some anorexic patients during refeeding. Because of the major role of beta-adrenoceptors in mediating sympathetic nervous system effects on energy metabolism, we are currently investigating cardiovascular, metabolic and neuroendocrine responses to the beta-adrenoceptor agonist isoproterenol in anorexia patients at low weight and following weight gain.

Preclinical studies in animals and pharmacologic studies in human subjects indicate that hypothalamic serotonin plays a major role in satiety responses to eating and in preference for high carbohydrate foods. Preliminary evidence suggests that bulimic anorexics, who characteristically binge and purge in addition to restricting food intake, have reduced central serotonin activity in comparison to patients who follow an eating pattern characterized exclusively by restriction of food intake. Our group has previously demonstrated that bulimic anorexics have reduced levels of post-probenecid cerebrospinal fluid 5-HIAA levels in comparison to restricting anorexics. We are presently conducting follow-up studies that examine the behavioral and neuroendocrine responses to the serotonin precusor L-tryptophan and the serotonin agonist M-chlorophenylpiperazine (in collaboration with Drs. D. Murphy and E. Mueller). Preliminary results of these studies will be analyzed during the upcoming year. A summary of recent neurotransmitter studies by the group is being presented as part of a two day symposium organized by the Section Chief to be held during an international psychiatric congress this year.

Neuroendocrine and neuropeptide studies. Previous investigations have demonstrated abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis in anorexia patients, with increased production and decreased clearance of cortisol. In our present studies (in collaboration with Dr. P. Gold), we have demonstrated increased urinary free cortisol excretion, resistance to dexamethasone-induced suppression of serum cortisol, and blunted corticotropin responses to corticotropin releasing hormone (CRF). Following weight recovery, urinary free cortisol excretion and dexamethasome response return to normal relatively promptly, while there is an apparent delay in normalization of the corticotropin response to CRF. Future studies will examine the relationship between these HPA axis abnormalities in anorexia and activity of norepinephrine and dopamine neurotransmitter systems.

The mechanism for altered activity of the hypothalamic-pituitary-thyroid (HPT) axis (with low circulating levels of triiodothyronine) in anorexia is poorly understood. Over the past year, we have found that the thyroid stimulating hormone (TSH) response to infusion of thyrotropin releasing hormone (TRH) is blunted and delayed in underweight anorexics. Following weight restoration in these patients, the TSH response returns to normal magnitude but remains delayed. The relationship between these HPT alterations and depressive symptoms is presently being evaluated, as well as the possible association between HPT activity and weight-related alterations in energy metabolism in anorexic patients.

Based on pharmacologic studies in laboratory animals and human subjects, it appears that the endogenous opiate system plays an important role in stimulating appetite and eating behavior. To assess the possibility that this system is dysregulated in anorexia nervosa, we have measured cerebrospinal fluid levels of pro-opiomelanocortin (POMC) related peptides. In underweight patients, levels of beta-endorphin (in collaboration with Dr. W. Berrettini), beta-lipotropin, ACTH (in collaboration with Dr. P. Gold), and the N-terminal fragment of POMC were all low in comparison to levels in age matched normal controls. Cerebrospinal fluid levels of these peptides in the anorexic patients returned to normal following weight restoration. Further analysis of these results should help clarify whether reductions in the POMC-related peptides are correlated with the severity of cognitive alteration, mood disturbance or body image distortion in the anorexic patients.

Energy metabolism and body weight regulation. The role of altered energy metabolism and weight regulation as premobid risk factors for anorexia nervosa is unknown. Moreover, the possible value of pharmacologic manipulation of energy metabolism in the treatment of anorexia has not been systematically explored. Previous studies suggest that caloric requirements for weight gain are greater in underweight anorexics than in cachectic non-psychiatric patients. Moreover, anorexic subjects with prior history of obesity have been reported to utilize ingested calories more efficiently than subjects without prior obesity. During the past year we have analyzed daily caloric intake for

anorexic patients at low weight and following weight restoration. These results showed that the daily caloric requirement for stable weight maintenance was significantly lower in underweight bulimic anorexics (874 kcal/day) than in food restricting patients (1037 kcal/day). A similar difference was observed between bulimic anorexics (1094 kcal/day) and restrictors (1590 kcal/day) following stable weight restoration. These results suggest that different symptom patterns between these two subgroups of anorexic patients may in part derive from different experiences in efforts to maintain a particular body weight.

Brain imaging studies. Preliminary analysis of computerized tomographic scans (in collaboration with Dr. C. Kellner) showed that underweight anorexic patients have increased venticular size as measured by both linear and planimetric determinations. Cortical atrophy was also apparent in this patient group, and was inversely correlated with body weight. There was a trend toward positive correlation between urinary free cortisol excretion and cortical atrophy, in parallel with an observation previously reported in depressed patients. Cortical atrophy in anorexia appears to be related to weight loss in that there was a tendency for return to normal neuroanatomic measures in patients studied following weight restoration.

Preliminary results for a small number of anorexic patients studied in a low weight cachectic state showed reduced utilization of ^{18}F-2-deoxyglucose, as measured by positron emission tomography (in collaboration with Dr. J. Winterer). Metabolic activity returned toward normal levels in patients studied following weight restoration. The initial scans are presently being analyzed for regional localization of the metabolic alterations.

B. Bulimia

Neurotransmitter turnover and receptor sensitivity. Altered norepinephrine has been indirectly implicated in symptoms of depression, anxiety and altered appetite, all of which are common in patients with bulimia. Since altered eating patterns per se can affect noradrenergic function, we have conducted studies soon after admission in an effort to evaluate the effects of bulimic ingestive patterns on norepinephrine activity. Other studies have been conducted after at least three weeks of stable meal patterns ("abstinence phase") to assess the underlying status of the noradrenergic system in these patients.

Systematic collection of blood samples during a naturalistic study of bingeing/vomiting behavior the day following hospital admission demonstrated substantial elevations of plasma norepinephrine. While this response pattern may simply reflect a dose-response relationship in the sense that large binge meals might be expected to produce larger catecholamine response than moderate-size test meals studied in healthy volunteers, it also suggests that a pattern of frequent (e.g., daily) bingeing might result in a functional resetting of norepinephrine responses in these patients.

When studied after at least three weeks' abstinence from bingeing, bulimic patients demonstrated lower resting plasma norepinephrine values and lower pulse rates than controls. Present studies do not clarify whether this down-regulation of sympathetic function is a persisting result of previous bulimic symptoms, or whether altered noradrenergic function may precede the onset of bulimia in these patients. Infusion studies with isoproterenol allowed us to compare beta-adrenoceptor sensitivity in bulimic patients and age-matched healthy controls. Bulimic patients showed greater sensitivity to isoproterenol-induced increases in heart rate than controls. In vitro measurement of lymphocyte beta-adrenoceptors (in collaboration with Drs. W. Potter and N. Buckholtz) showed a tendency toward increased receptor density in comparison to control values. Taken as a whole, these results suggest that decreased basal levels of circulating plasma catecholamines in bulimic patients result in up-regulation of beta-adrenoceptor responsiveness. Such receptor changes could render these patients more vulnerable to the consequences (e.g., anxiety) of stress-induced catecholamine release.

Previous studies have associated decreased central serotonin function with impaired satiety responses, depressive symptoms, impulsive behavior, and therapeutic response to antidepressant drugs, all of which are observed in bulimia. Studies in progress to assess central serotonin function in bulimic patients focus on challenge studies with the serotonin precursor L-tryptophan and the serotonin receptor agonist m-chlorophenylpiperazine (in collaboration with Drs. D. Murphy and E. Mueller). Behavioral and neuroendocrine responses will be compared in patients and healthy controls.

Neuroendocrine and neuropeptide studies. Evaluation of the HPA axis in bulimic patients (in collaboration with Dr. P. Gold), has demonstrated normal excretion of urinary free cortisol, but significant decrease in the ability of dexamethasome to suppress plasma cortisol. This blunted response to dexamethasone was observed in both post-admission and abstinent phases, and was not significantly related to presence or absence of a concomitant major depressive episode. This result is of particular interest in that it further highlights the lack of syndromal specificity for dexamethasone non-suppression of plasma cortisol.

Studies of the HPT axis in abstinent bulimic patients have demonstrated significantly reduced plasma levels of triiodothyronine, and blunted, delayed TSH responses to TRH. The possible impact of these thyroid abnormalities on body weight regulation in bulimia will be assessed in future studies of energy metabolism in this patient group.

Previous preliminary reports have suggested that serum amylase was elevated in bulimic patients. We have measured amylase levels in systematically collected blood samples from restrictor anorexic, bulimic anorexic, normal weight bulimic and heathy control subjects. Admission amylase levels were significantly higher in the bulimic patient groups.

Blood samples collected from bulimic patients following bingeing and vomiting showed four fold increases in serum amylase levels, whereas consumption of a large meal did not significantly alter serum amylase in healthy volunteers. Studies presently in progress will help to clarify whether the elevated amylase levels in bulimic patients derive primarily from the pancreas or salivary glands. Further analysis of binge-induced amylase elevations will help to clarify the value of serum amylase determination as a screen for recent bingeing/vomiting episodes in bulimic patients.

Behavioral responsiveness to metabolic challenge with double-blind sodium lactate infusion was compared in bulimic patients and controls. This challenge procedure results in panic attacks in a majority of patients with panic disorder. Since bulimic patients and their first degree relatives have significantly increased lifetime prevalence of anxiety disorders, several groups have questioned whether binge episodes in bulimic patients might be biologically related to panic attacks. Utilizing lactate infusion as a challenge to test this proposal, we found increased baseline anxiety ratings in bulimic patients, but no evidence of increased sensitivity to anxiety producing effects of lactate.

Psychological studies. Ratings of anxiety and depression were obtained during a naturalistic study of binge/vomiting episodes as part of the admission assessment of bulimic patients. These ratings substantiated other reports of decreased anxiety following binge episodes; decreases in anxiety ratings were observed more consistently than reduced depression ratings.

Data collection was completed for a study that compared psychological characteristics of restricter anorexic, bulimic anorexic, normal weight bulimic and control groups. Across eight psychometric scales, level of psychopathology for normal weight bulimics was similar to that for the anorexic groups. Bulimic subjects had relatively high scores on anger, hostility and impulsivity scales.

Significance to Biomedical Research and the Program of the Institute:

The eating disorders of anorexia nervosa and bulimia occur in five to ten percent of high school and college age women. Despite their relatively high prevalence, there are limited data available on the psychobiology of these disorders. The studies described above further clarify the involvement of the noradrenergic neurotransmitter system in these disorders. The neuroendocrine findings have a number of similarities to results reported in depressed patients, and emphasize the possibility of underlying biological similarities across these distinct clinical syndromes. The results suggest possible new treatment strategies directed toward neurotransmitter, neuroendocrine and neuropeptide systems.

Proposed Course:

We are beginning a set of studies to evaluate more carefully changes in noradrenergic function across different phases of symptomatology and recovery in the eating disorders, with particular attention to effects on energy metabolism (assessed by indirect calorimetry) and interaction with hormonal neuromodulators (e.g., thyroid hormones). We are conducting pharmacologic studies of the serotonin system in relationship to patterns of altered appetite and mood regulation. We have established an eating disorder outpatient clinic to assess neurotransmitter and neuroendocrine function in bulimic patients less severely symptomatic than the inpatient population. Present therapeutic studies in bulimic outpatients will assess the clinical efficacy of serotonin-active drugs in this population. A family study (in collaboration with Dr. E. Gershon) has been initiated to assess the prevalance of depression and other major psychiatric syndromes in first degree relatives of bulimic patients. Behavioral, neuroendocrine and metabolic responses to 2-deoxyglucose-induced intracellular glucose deprivation (in collaboration with Drs. S. Paul, D. Pickar and O. Wolkowitz) are being assessed in eating disorder patients. Follow-up studies on the endogenous opiate system and gut-related peptide hormones will involve further cerebrospinal fluid studies and measurement of peripheral hormone responses to physiologic challenge procedures.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00326-12 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Neuropharmacology and Psychobiology of Depression and Mania

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dennis L. Murphy, M.D., Chief
Section on Clinical Neuropharmacology, LCS, NIMH

COOPERATING UNITS *(if any)*

BP, CPB, LCS, NIMH; USUHS, VA Med. Cen., Bronx, NY

LAB/BRANCH

Laboratory of Clinical Science

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.0	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of a systematic comparison of substrate-selective monoamine oxidase (MAO)-inhibiting antidepressants, a dose-response study of deprenyl was completed this year. Minimal antidepressant effects were obtained at an MAO-B selective deprenyl dose of 10 mg/day, which inhibited platelet MAO activity greater than 95%. With higher doses (30 and 60 mg/day), reductions in plasma MHPG approached those found with non-selective inhibitors or the MAO-A selective inhibitor, clorgyline. Moreover, a markedly increased pressor sensitivity to intravenous tyramine occurred with the higher deprenyl doses. These results add further support to our hypotheses that both antidepressant activity and the potentiation of tyramine sensitivity are most closely linked with MAO-A inhibition and changes in noradrenergic functions. In animal studies of two new MAO-A selective reversible inhibitors, cimoxatone most closely resembled clorgyline in its effects on monoamine metabolism, while amiflamine had relatively greater effects on serotonin metabolism than did clorgyline or cimoxatone. Amiflamine, however, also possessed monoamine-releasing effects, complicating its use in studying the consequences of selective MAO-A inhibition.

PHS 6040 (Rev. 1/84) GPO 914-918

Other collaborative professional personnel engaged on the project:

C. S. Aulakh	Visiting Associate	LCS, NIMH
B. Roy	Staff Psychiatrist	LCS, NIMH
L. J. Siever	Staff Psychiatrist	VA Med. Ctr., Bronx
E. A. Mueller	Staff Fellow	LCS, NIMH
T. Sunderland	Staff Fellow	LCS, NIMH
R. M. Cohen	Staff Psychiatrist	LCS, NIMH
T. Uhde	Staff Psychiatrist	BPB, NIMH
M. Linnoila	Clinical Director	ALC IR, NIAAA
L. F. Major	Staff Psychiatrist	Upstate Med. Ctr. SUNY - New York
D. M. Kuhn	Senior Investigator	HE, NHLBI
N. A. Garrick	Biologist	LCS, NIMH

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with characterologic disorders, those with depression secondary to other psychiatric disorders (e.g. personality disorders) and neurologic disorders (e.g. Parkinson's disease, Alzheimer's dementia), are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patients' self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed in this Branch.

2. Biological assessment: Pharmacologic challenge tests of central neurotransmitter systems are used to evaluate the functional state of these systems in patients compared to controls, and in patient groups studied before and during antidepressant drug treatment. Neuroendocrine, cardiovascular and behavioral changes are used as endpoints in these studies. Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of biogenic amines and their metabolites, enzymes, other chemical variables, and drug levels.

Major Findings:

To gain a better understanding of the mode of action of monoamine oxidase (MAO)-inhibiting antidepressants, we have compared the consequences of selective MAO-B inhibition by deprenyl with our previous findings with the MAO-A selective inhibitor, clorgyline.

A major problem with MAO inhibitors is their potentiation of tyramine in foodstuffs and interactions with tyramine-like drugs. Deprenyl has previously been reported to be a selective monoamine oxidase (MAO) type B inhibitor, which is associated with little or no enhancement of the pressor effects of tyramine. Employing an intravenous steady-state tyramine infusion technique, the effects of different doses of deprenyl and, for comparison, the mixed inhibitor tranylcypromine on the pressor response to tyramine were studied in 11 depressed patients. After 3 weeks of treatment, deprenyl produced

dose-proportionate increases in tyramine sensitivity at all three doses (10, 30, and 60 mg/day) when compared to placebo baseline tyramine responses. While only a modest (3.7-fold) increase in tyramine sensitivity was found with the 10 mg/day deprenyl dose, the increase in tyramine sensitivity at the 60 mg/day dose of deprenyl (22-fold) approached that found with the clinically-used MAO-inhibiting antidepressant, tranylcypromine.

Reductions in plasma 3-methoxy,4-hydroxyphenylglycol (MHPG), used as a possible index of *in vivo* MAO-A inhibition, were highly correlated with increases in tyramine pressor sensitivity (r=0.82). These data suggest that deprenyl acts as a relatively selective MAO-B inhibitor at low doses, but that this selectivity is lost at higher doses, resulting in a significant "crossover" inhibition of MAO-A and increased tyramine pressor sensitivity. As only minimal antidepressant effects were observed with deprenyl except at the highest doses studied, the deprenyl data add further support to our hypotheses that both antidepressant activity and the potentiation of tyramine sensitivity are most closely linked with MAO-A inhibition and changes in noradrenergic function.

In animal studies, we have compared two new, reversible MAO-A selective inhibitors with the irreversible MAO-A inhibitor, clorgyline. Acute treatment with the reversible inhibitor cimoxatone (0.5-8 mg/kg) resulted in generally dose-dependent reductions in cerebrospinal fluid concentrations of MHPG, 5-HIAA and HVA of 20-50%, 7-25% and 27-50%, respectively, compared to baseline. A larger reduction in MHPG (52 ± 1%) was obtained following 8 mg/kg cimoxatone, while reductions in 5-HIAA (25 ± 5%) and HVA (36 ± 5%) were not proportionately larger at this dose.

Amiflamine over the dose range studied (0.48-5.77 mg/kg) produced smaller changes in CSF metabolites which peaked in the first 12 hours, perhaps reflecting the shorter half-life of this reversible inhibitor. At these doses, MHPG was reduced 4-26%, 5-HIAA 1-28% and HVA 7-29%. Overall, maximum reductions followed treatment with 1.44 mg/kg, with MHPG, 5-HIAA and HVA being decreased 21 ± 2%, 28 ± 2% and 29 ± 3%, respectively. This dose is similar to the maximum dose (100 mg) studied in man. Initial transient but marked increases in 5-HIAA and HVA concentrations compared to baseline were also observed with amiflamine at some doses. Although CSF amine concentrations have not yet been measured, these data suggest an amine-releasing effect of amiflamine within serotonin and possibly dopamine neurons.

Irreversible inhibition of MAO-A following clorgyline treatment (1-2 mg/kg) resulted in dose-dependent reductions of 50-68% in MHPG, 7-28% in 5-HIAA and 22-48% in HVA. At 1 mg/kg, MHPG was reduced 50 ± 8%, while HVA and 5-HIAA were decreased 22 ± 5% and 7 ± 3%, respectively. Chronic clorgyline administration at doses (0.25 - 0.5 mg/kg/d, 24d, im) similar to those used in man for antidepressant treatment (0.35 mg/kg), resulted in an MHPG reduction from baseline (68 ± 3%) equivalent to that seen with acute clorgyline at 2 mg/kg; however, changes in HVA (14 ± 2%) and 5-HIAA (0 ± 4%) were less than those resulting from acute clorgyline treatment.

These results indicate that the reversible inhibitor cimoxatone, at higher doses, produces CSF metabolite reductions which approach those obtained with the irreversible MAO-A inhibitor clorgyline. At mid-range doses, cimoxatone and amiflamine have lesser effects on MHPG and greater effects on 5-HIAA and (for cimoxatone) on HVA than clorgyline. Amiflamine, in addition, appears to possess more complex amine-releasing properties which require further study.

Significance to Biomedical Research and the Program of the Institute:

These new data provide further physiological, behavioral and neurochemical evidence for differential consequences of the inhibition of MAO-B versus MAO-A in humans and animals.

The ability to continuously monitor changes in the central metabolism of catecholamines and indoleamines following acute and chronic MAO inhibition to nonhuman primates provides valuable information not readily attainable in human clinical trials, and leads to a more complete understanding of MAO-inhibiting drugs.

Proposed Course:

We are extending the clinical studies of selective MAO-inhibiting drugs to other patient populations under investigation in the section. Only a handful of depressed patients have been admitted to our inpatient unit this past year, and, except for some continuing studies of older depressed patients, we plan to continue to limit our affective disorder-related studies to allow more in-depth investigation of the affective components of other disorders covered under separate reports, i.e., Alzheimer's disease and obsessive-compulsive disorder.

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Garrick, N.A., Seppala, T., Scheinin, M., Chang, W.-H., Linnoila, M., and Murphy, D.L.: Differential changes in rhesus monkey cerebrospinal fluid amine metabolites following reversible and irreversible monoamine oxidase type A inhibitors. In Tipton, K.F., Dostert, P., Strolin-Benedetti, M., Dollery, C.T., Fowler, C.J., and Zarifian, E. (Eds.): Monoamine Oxidase and Disease: Prospects fr Therapy with Reversible Inhibitors. New York, Academic Press, 1985, pp. 604-605.

Siever, L.J., Uhde, T.W., Potter, W.Z., and Murphy, D.L.: Norepinephrine in the affective disorders. Receptor assessment strategies. In Lake, C.R. and Ziegler, M.G. (Eds.): The Catecholamines in Psychiatric and Neurologic Disorders. Stoneham, MA, Butterworth Publishers, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00329-10 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Platelets and Other Systems as Models for the Study of Neurotransmitter Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jonathan L. Costa, Staff Physician LCS NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was discontinued following the departure of the principal investigator from NIMH in July, 1984.

Publications:

Corash, L., Costa, J.L., Shafer, B., Donlon, J.A., and Murphy, D.L.: Heterogeneity of human whole blood platelet subpopulations. III. Density dependent differences in subcellular constituents. Blood 64: 185-193, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00330-07 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Electron and Photon Imaging Techniques to Study Aminergic Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jonathan L. Costa, Staff Physician LCS NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was discontinued following the departure of the principal investigator from NIMH in July, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00331-07 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Use of Nuclear Magnetic Resonance to Study Aminergic Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jonathan L. Costa, Staff Physician LCS NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was discontinued following the departure of the principal investigator from NIMH in July, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00332-07 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal Models for the Study of Neuropharmacologic Effects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Charanjit S. Aulakh Visiting Associate LCS NIMH

COOPERATING UNITS *(if any)*

LCM, NIMH; Howard University

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The behavioral as well as neurochemical effects of long-term administration of the tricyclic antidepressant desipramine and the selective monoamine oxidase (MAO) type A-inhibiting antidepressant clorgyline were studied in rats. As previously observed with clorgyline, long-term but not short-term desipramine administration attenuated the suppressant effect of clonidine on self-stimulation behavior, demonstrating adaptive changes in the noradrenergic system with both antidepressants. Failure of long-term clorgyline treatment to down-regulate α_1, α_2 and β-adrenergic receptor densities in 6-hydroxydopamine lesioned animals further demonstrates that adrenergic receptor adaptation to MAO inhibitors is a response to an increase in catecholamine receptor occupancy. In another study, continuous administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) via osmotic minipumps to rats was shown to produce sustained locomotor deficits which might be related to the movement disorders (akinesia) of Parkinson's disease.

Other collaborative professional personnel engaged on the project:

D. L. Murphy	Chief	LCS NIMH
R. M. Cohen	Section Chief	LCM NIMH
S. N. Pradhan	Professor	Howard University
K. M. Wozniak	Visiting Fellow	LCS NIMH
N. Zamir	Visiting Associate	LCS NIMH

Objectives

The latency in the onset of the clinical effects of antidepressant drugs has prompted investigators to study the slowly developing behavioral, biochemical and electrophysiological changes in the central nervous system following long-term administration of these drugs in animals. These slowly developing changes might be important in understanding the molecular mechanisms responsible for both the therapeutic and the side effects of these drugs. With this approach in mind we have been investigating the adaptive changes in both the noradrenergic and serotonergic neurotransmitter mechanisms following long-term administration of monoamine oxidase inhibitors and tricyclic antidepressants since both of these neurotransmitter mechanisms have been implicated in the etiology of affective disorders.

Methods Employed:

For studies of self-stimulation behavior, bipolar stainless steel electrodes are implanted stereotaxically in different brain regions. A week after surgery, the animals are trained to press a lever in a Skinner box in order to receive reinforcement from intracranial electrical stimulation.

Locomotor activity of individual rats is measured in activity cages equipped with photocells. Interruption of photobeams is recorded automatically by digital counters.

Food and water intake (24 h) of individual rats is measured daily.

Receptors from crude brain homogenates are measured by standard radioactive ligand assays. [^{3}H]-WB4101, [^{3}H]-clonidine and [^{3}H]-dihydroalprenolol are the specific ligands used for the measurement of α_1-α_2- and β-adrenoreceptors, respectively. The concentrations of norepinephrine, dopamine and serotonin are determined by high pressure liquid chromatography with electrochemical detection.

Major Findings:

Long-term administration of the tricyclic antidepressant desipramine (10 mg/kg/day) did not change the rate of self-stimulation responding in the A10 (ventral tegmental) area but significantly attenuated the suppressive effect of the selective α_2-adrenergic agonist clonidine on this behavior. These findings demonstrate an involvement of noradrenergic mechanisms in the regulation of ventral tegmental self-stimulation and further suggest that adaptive changes in the inhibitory presynaptic noradrenergic receptors may be involved in desipramine's antidepressant effects.

Long-term administration of clorgyline (1 mg/kg/day), a selective monoamine oxidase type A inhibiting antidepressant, increased norepinephrine (NE) and serotonin (5HT) levels and caused a decrease in the densities of α_1-, α_2- and β-adrenergic receptors in the cortex. 6-Hydroxydopamine lesions attenuated clorgyline's effect on cortical NE levels but not 5HT, and, furthermore, effectively blocked the decrease in α_1, α_2- and β-adrenergic receptor densities observed in response to clorgyline treatment.

The continuous infusion of the parkinsonian syndrome-producing neurotoxin, MPTP (10-40 mg/kg/day for 14 days) via osmotic minipumps implanted subcutaneously produced dose-dependent decreases in locomotor activity and food and water intake in rats. Following discontinuation of MPTP, food and water intake returned towards pretreatment values whereas locomotor deficits persisted for at least seven days.

Significance to Biomedical Research and the Programs of the Institute:

The functional adaptational changes in the noradrenergic pathways following long-term treatment with an antidepressant drug detected using a self-stimulation paradigm are important since this neurotransmitter mechanism has been implicated in the etiology of the affective illnesses. It has been proposed that depression may result from a pathological hypoactivity of a reward system in the brain which uses a catecholamine as its neurotransmitter. Also, the present data demonstrating attenuation of clorgyline's effects on cortical norepinephrine levels and α_1, α_2- and β-adrenergic receptor densities in the 6-hydroxydopamine lesioned animals suggest that β-adrenergic receptor adaptation to MAO inhibitors as with tricyclic antidepressants is a response to an increase in catecholamine receptor occupancy, and that a similar molecular mechanism is responsible for the observed clorgyline-induced changes in α-adrenergic receptors.

The demonstration of sustained locomotor deficits in rats following discontinuation of MPTP in this study might ultimately be helpful in understanding the akinesia associated with Parkinson's Disease.

Proposed Course:

During the next year, we will be attempting to explore functional adaptational changes in the serotonergic system using different animal behavioral models. Also, we would like to examine the interrelationship between the serotonergic and noradrenergic systems in adaptational processes accompanying long-term antidepressant drug administration.

We shall also continue to investigate the persistence of the MPTP-induced behavioral effects in rats for longer time periods. In addition, we shall attempt to elucidate the neurochemical bases of these behavioral changes.

Publications:

Murphy, D.L., Garrick, N.A., Aulakh, C.S. and Cohen, R.M.: New contributions from basic science to understanding the effect of monoamine oxidase inhibiting antidepressants. J. Clin. Psychiatry 45: 37-43, 1984.

Aulakh, C.S., Cohen, R.M., Pradhan, S.N. and Murphy, D.L.: Long-term desipramine treatment attenuates clonidine-induced suppression of ventral tegmental self-stimulation. Life Sci. 36: 443-447, 1985.

Wozniak, K.M., Aulakh, C.S. and Murphy, D.L.: Sustained reductions in locomotor activity in rats following MPTP treatment using osmotic minipumps. In Markey, S.P., Castagnoli, N., Kopin, I.J., and Trevor, A.J. (Eds.): MPTP-A Parkinsonian Syndrome Producing Neurotoxin. Academic Press, New York.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00336-06 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Phenomenology and Treatment of Obsessive-Compulsive Disorder in Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.R. Insel	Staff Physician	LCS, NIMH
Others:	D.L. Murphy	Chief	LCS, NIMH
	J. Zohar	Visiting Associate	LCS, NIMH
	E.A. Mueller	Staff Physician	LCS, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Clinical Studies, NIAAA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.8	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Obsessive-compulsive disorder has been studied from several different perspectives since the beginning of this project in 1980. During the past year, three areas of interest have been pursued. We have followed up on our previous treatment studies with a double-blind comparison of the tricyclic antidepressants clomipramine and desipramine. Clomipramine appears to be more effective than desipramine for reducing obsessional symptoms, although the drugs have been previously shown to be equally effective as antidepressants. A second research focus has looked at cerebral blood flow during laboratory precipitated anxiety in obsessive-compulsive disorder patients. Results from this study are currently being analyzed. Finally, the natural course of this disorder has been investigated with a follow-up study of subjects recruited in 1980 and 1981.

Other Collaborative Professional Personnel Engaged on the Project:

M. Linnoila	Section Chief	LCS, NIAAA

Project Description:

Objectives: This is the sixth year for this project investigating the psychobiology of obsessive-compulsive disorder. The first three years were dedicated to a wide-ranging series of investigations of the phenomenology, neuropsychology, and pharmacologic treatment of this disorder. A major finding from these studies was that the tricyclic antidepressant clomipramine was more effective than the MAO inhibitor clorgyline in reducing obsessional symptoms. In a follow-up of this treatment study, two neurochemically selective antidepressants (zimelidine and desipramine) were studied, but neither appeared effective. In the past year, we have more closely examined if clomipramine is, in fact, selectively reducing obsessional symptoms or if the structurally related tricyclic anti-depressant desipramine might be equally effective. As clomipramine and desipramine are roughly equipotent as antidepressants, the discovery of a difference in their anti-obsessional potency would confirm our earlier impression that clomipramine has specific anti-obsessional properties.

We undertook three other objectives in this past year. First, consistent with our interest in the neurobiology of anxiety (see Project No. Z01 MH 02219-02 LCS) we began to study physiologic responses of compulsive washers challenged with their phobic stimuli. The goal of this study was to investigate cerebral blood flow and peripheral autonomic changes with behaviorally induced anxiety in the laboratory. Two other facets of this project which began this year were (1) a follow-up of our early cohort of obsessive-compulsive disorder patients to quantitatively define the natural course of this disorder and (2) the development of new criteria for obsessive-compulsive disorder in collaboration with committees for the revision of DSM-III and ICD-9.

Methods Employed: Treatment studies are conducted in the NIMH Outpatient Clinic at the Clinical Center. Local patients with obsessive-compulsive disorder are accepted if they have been ill for at least one year and are willing to stop all psychotropic medication. Following two to four weeks of placebo administration, patients are randomly assigned to double blind treatment with either desipramine (300 mg) or clomipramine (300 mg). After five weeks, the first drug is replaced by placebo for two to four weeks prior to crossover to the second drug. Patients are assessed weekly throughout the study with a battery of psychological ratings.

Cerebral blood flow studies are completed in the Behavioral Neurology Laboratory at St. Elizabeths Hospital using the ^{131}Xe inhalation method. Each patient is studied when drug free. Following two hours of adaptation to the apparatus, the patient enters three carefully controlled protocols. In the first test he listens to a relaxation tape for a "resting" condition. In the second test an "imaginal exposure" tape describes the phobic situation. In the final "in vivo" exposure condition, the patient touches the phobic stimulus while listening to the exposure tape. The patient keeps his eyes closed in all three protocols. In addition to cerebral blood flow, heart

rate, blood pressure, galvanic skin response, and subjective anxiety are monitored throughout the study (including during the adaptation period).

Major Findings: Ten patients have completed the desipramine-clomipramine crossover study so far. Preliminary results suggest that desipramine is not generally effective for obsessive-compulsive patients, although one patient markedly improved while on the drug. Clomipramine continues to reduce obsessional ratings about 40% with six patients showing marked improvement during the double blind treatment. These data, while still very preliminary already suggest a difference in anti-obsessional effectiveness between the two drugs. Unfortunately, clomipramine administration is associated with a number of adverse effects which may limit its long term use.

Cerebral blood flow data are not yet available. With the first eight patients who have completed the study, we have demonstrated that obsessional patients develop intense anxiety in the laboratory setting. Subjective ratings of anxiety increased from 3.0 units during relaxation to 7.2 units during the "in vivo" exposure condition. Peripheral autonomic changes generally followed the increases in subjective discomfort (heart rate increased 11.5 bpm) except for one patient who developed bradycardia and hypotension when anxious.

Significance to Biomedical Research and the Program of the Institute: These studies of obsessive-compulsive disorder continue a program of research into an important and poorly understood psychiatric syndrome. The importance of this syndrome resides not only in its debilitating and treatment refractory nature, but in its unique intermingling of affective and cognitive symptoms and in its highlighting intrapsychic conflicts over aggressive impulses. By establishing a focus of interest in this disorder, the NIMH has become one of the major referral centers for obsessional patients from across the country.

Proposed Course: During the coming year we plan to complete the clomipramine-desipramine comparison. In addition, we hope to focus more on the mechanism of clomipramine's effects by using challenge tests with the serotonergic receptor agonist m-CPP to assess changes in post-synaptic receptor sensitivity following clomipramine administration.

Our initial experiences with the cerebral blood flow study have encouraged us to use obsessional patients to learn more about physiologic changes during behaviorally evoked anxiety. We hope to extend this paradigm to study changes in peripheral catecholamines and hormone levels and to document the effects of anti-obsessional drugs such as clomipramine.

A major focus in the coming year will be the follow-up assessment of our original cohort of obsessive-compulsive patients. By repeating the same assessment measures and gathering a careful history, we will be able to define the course of this disorder and evaluate the ultimate effects of pharmacologic treatment.

Publications:

Insel, T.R., Mueller, E.A., Gillin, C., Siever, L.J., and Murphy, D.L.: Biological markers in obsessive compulsive and affective disorders. J. Psychiatr. Res. 18: 407-423, 1984.

Insel, T.R.: Obsessive compulsive disorder. Psychiatr. Clin. N. Amer. 8: 105-119, 1985.

Insel, T.R., and Mueller, E.A.: Obsessive compulsive disorder: Pharmacologic approaches. In Pichot, P., Berner, P., Wolf, R., and Thau, K. (Eds.): Psychiatry. New York, Plenum Publishing Corporation, 1985, Vol. 4, pp. 259-263.

Insel, T.R., Mueller, E.A., Gillin, C., Siever, L.J., and Murphy, D.L.: Tricyclic response in obsessive compulsive disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry 9: 25-31, 1985.

Insel, T.R., Mueller, E.A., Murphy, D.L., Linnoila, M.: Obsessive compulsive disorder and serotonin: Is there a connection? Biol. Psychiatry (in press).

Insel, T.R., Zahn, T., and Murphy, D.L.: Obsessive compulsive disorder: An anxiety disorder. In Mazur, J. and Tuma, H. (Eds.): Anxiety and Anxiety Disorders. Englewood Cliffs, NJ, Lawrence Earlbaum Press (in press).

Zohar, J., and Insel, T.R.: Drug treatment of obsessive compulsive disorder. Psychiatr. Med. (in press).

Zohar, J., and Insel, T.R.: Obsessive compulsive disorder and alcoholism. J. Clin. Psychiatry (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00337-06 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropharmacology of Neuroendocrine and Neurotransmitter Regulatory Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dennis L. Murphy, M.D. Chief, Section on Clin. Neuropharmacology LCS NIMH

COOPERATING UNITS *(if any)*

Centre for Reproductive Biology, Edinburgh, Scotland; LN, CPB, NIMH; NIAAA; Department of Medicine, University of California, San Diego

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the first study in humans of the selective serotonin receptor agonist, m-chlorophenylpiperazine, significant neuroendocrine, behavioral and temperature changes were observed to follow oral administration of 0.5 mg/kg of the drug in 14 normal volunteers.

Nocturnal elevations in N-acetyl-serotonin (but not in serotonin) were found to be associated with the diurnal melatonin rhythm in rhesus monkey cerebrospinal fluid.

Evidence that dopamine and L-dopa may produce their cardiovascular effects in humans via the production of norepinephrine and epinephrine was obtained; in contrast, dopamine agonists such as bromocriptine reduced plasma human norepinephrine concentrations.

Physiologic factors controlling the release of atrial natriuretic peptide (ANP) from rodent heart were evaluated using a newly-developed radioimmunoassay. Basal levels of this peptide in conscious rats were found to be considerably lower than those previously reported in rats studied under anesthesia. Fluid volume loading using saline or glucose increased plasma ANP concentrations 4-5 fold; similar effects did not occur in rats studied during halothane anesthesia or in pithed rats, raising the possiblity of CNS neural or hormonal influence on ANP release.

PHS 6040 (Rev. 1/84) GPO 914-918

Other collaborative professional personnel engaged on the project:

N. A. Garrick	Biologist	LCS NIMH
S. P. Markey	Section Chief	LCS NIMH
M. Mishkin	Chief	LN NIMH
E. A. Mueller	Senior Staff Fellow	LCS NIMH
T. Sunderland	Senior Staff Fellow	LCS NIMH
L. Tamarkin	Staff Fellow	CPB NIMH
P. Taylor	Chemist	Centre for Reproductive Biology, Edinburgh
N. Zamir	Visiting Associate	LCS NIMH
M. G. Ziegler	Assoc. Professor	University of California, San Diego

Project Description:

Objectives: The discovery that many newly-discovered peptides and hormones are present in high concentrations in the brain, cerebrospinal fluid (CNS) and plasma has led to an entire field of inquiry into the modulatory interactions between peptides, hormones and both the classical monoamine neurotransmitters as well as trace amines in brain. All of these substances may function as modulators of neurotransmission. This project has focused on the measurement of various peptides, hormones and several monoamines and their metabolites in cerebrospinal fluid and plasma in an attempt to evaluate (a) CNS physiologic influences on hormones and monoamines (e.g. diurnal and light-regulated changes); (b) the relationship between plasma and CSF peptide levels; and (c), in particular, to assess the effects of drugs, stress, and other stimuli which are known to affect monoamines, peptides and hormones using biochemical, behavioral and neuroendocrine response measures.

Methods Employed:

Human plasma is obtained from blood samples collected via indwelling venous catheters. Cerebrospinal fluid from non-human primates is collected by means of indwelling lumbar or lateral ventricular cannulae for continuous flow through a refrigerated line into a fraction collector housed in a freezer. Plasma from the non-human primates and rodents is obtained by use of indwelling venous catheters which are usually implanted 15 to 24 hours prior to our studies to permit investigations under non-stressful, basal conditions. Some examples of hormones measured by radioimmunoassay include cortisol, prolactin, growth hormone, beta-endorphin, melatonin and several others, including a series of assays for atrial natriuretic peptides developed this year by Dr. Zamir. Serotonin and N-acetyl-serotonin are measured by capillary mass spectrometry. Monoamine metabolites are measured by high performance liquid chromatography with electrochemical detection.

Major Findings:

Preliminary dose-response studies in humans revealed that 0.5 mg/kg m-chlorophenylpiperazine (m-CPP) was well tolerated and produced reliable neuroendocrine responses. In a placebo-controlled, double-blind evaluation of the effects of this serotonin receptor agonist, statistically significant three-fold increases in plasma prolactin and two-fold increases in plasma cortisol were found in a group of 14 normal subjects. Small but statistically-significant increases in self-rated activation/euphoria and anxiety and in body temperature also followed m-CPP administration. No cardiovascular changes were observed.

Dopamine (2-4 ng/kg/min, iv) and L-dopa (500 mg, po) enhanced cardiovascular function both via direct effects of dopamine as well as by their conversion to norepinephrine and epinephrine. In contrast, the dopamine agonist, bromocriptine (60 mg, po), significantly reduced plasma norephinephrine and decreased systolic and diastolic blood pressure while apomorphine (0.75 mg subcutaneously) produced minimal catecholamine and cardiovascular changes.

Night time elevations in N-acetyl-serotonin (NAS) were found in the cerebrospinal fluid of rhesus monkeys. Improved GC-MS assay methodology permitted separate evaluations of NAS and serotonin, and clarified that the diurnal rhythms in NAS rather than those of serotonin parallel those of melatonin in cerebrospinal fluid.

A radioimmunoassay for atrial natriuretic peptide (ANP) based upon an antiserum against alpha-rat ANP_{5-28} was successfully developed. On the basis of cross-reactivity studies, this antiserum appears to be both conformational and mid-portion directed. It recognizes various ANP's as follows: alpha-$RANP_{3-28}$ (ANF_{8-33}), alpha-$RANP_{4-28}$ (Auricculin B), and alpha-$RANP_{5-28}$ (ATP III), alpha-$RANP_{5-27}$ (ATP II), and alpha-$RANP_{5-25}$ (ATP I) on an equimolar basis. In contrast, it binds to alpha-$RANP_{4-24}$ (Auriculin A) with a 2.5-fold greater avidity than ATP III and it recognizes alpha-$RANP_{1-28}$ (ANF) and alpha-human ANP_{1-28} only 10% and < 0.2% as well as ATP III, respectively. The characteristics of this antiserum in a routine overnight RIA reveal a maximum sensitivity of 3 picograms per tube, with an intra-assay sample variation of 3% and an inter-assay sample variation of 5%.

To study physiologic factors regulating ANP release, chronic indwelling catheters were placed in the femoral vein and artery of adult male rats 24 hours prior to the experimental manipulations. IR-ANP levels in conscious, unrestrained control animals with indwelling catheters were found to be 125 $\pm$ 15 or 80 $\pm$ 10 pg/ml in two independent experiments. These measurements of basal ANP levels in conscious rats constitute the first report of IR-ANP levels in non-anesthetized, non-traumatized animals and with one exception were found to be one-fifth to one-fifteenth of previously reported values. Volume loading with either 5% glucose or 0.9% saline enhanced plasma levels of IR-ANP 4-5-fold. Hyperosmotic challenge using hypertonic saline also resulted in a rapid increase in plasma ANP similar to that observed following volume loading.

Clearly, volume expansion or hyperosmotic challenge is a rapid, potent inducer of ANP release; however, whether or not this release is due to a direct effect on the ANP-containing cardiocytes or via an indirect reflex mechanism involving the central nervous system is not known. In an attempt to clarify the role of a neuronal reflex versus a direct cardiac effect as an explanation for observed volume load-induced release of ANP, groups of animals were anesthetized with halothane, followed by either bilateral vagotomy or sham vagotomy and volume load. Exposure to halothane for 15 minutes resulted in a 3-fold increase in plasma ANP. Vagotomy in halothane-anesthetized rats did not further alter circulating ANP levels or modify volume load-induced release of ANP. Halothane anesthesia itself blocked the volume load-induced release of ANP. Since the site of action of volume load-induced release of ANP is not known, whether or not the blocking action of halothane occurs directly at the myocardial level or indirectly via alterations of neuronal or hormonal input to the heart is not clear. Complete cardiac denervation in the pithed-rat preparation, which removes both humoral influences of central nervous system origin and direct neuronal control of the heart via the vagal and sympathetic nerves, blocked the volume load-induced release of ANP.

Significance to Biomedical Research and the Program of the Institute:

Serotonin, melatonin and related indoleamines participate in the regulation of sleep, locomotor activity, reproductive function and may influence several different hormones, including cortisol and prolactin. Abnormalities in these functions are found in depression and some other psychiatric disorders. The neuroendocrine, temperature and behavioral responses found in the first study in humans using the serotonin receptor agonist, m-CPP, further advance our hopes that this agent may be of value as a probe of the status of central serotonin receptors in various psychiatric disorders and during treatment with antidepressant and other drugs thought to act, in part, via serotonergic mechanisms. The cardiovascular and catecholamine changes found with dopaminergic agents complement our previous studies evaluating these substances as possible probes of central and peripheral catecholamine responsivity. Studies of monoamines in cerebrospinal fluid offer a direct approach to complement indirect neuroendocrine challenge studies with monoamine agonists in humans. Investigations of peptides involved in cardiovascular function may prove relevant to the effects and side-effects of psychoactive drugs.

Proposed Course:

Based on our studies with m-chlorophenylpiperazine in rodents, monkeys and now in normal humans, we plan to use this central serotonin agonist as a probe to evaluate evidence from other study approaches suggesting abnormalities in serotonin function in various psychiatric disorders. We also plan to use this agent to assess the possible involvement of serotonin receptor changes in the mode of action of antidepressants and other psychoactive drugs in animals and humans. Further information regarding the specificity of m-chlorophenylpiperazine for serotonergic receptors will be sought through studies with serotonin antagonists, through the use of agents known to alter brain serotonin receptors in animals, and through measurements of additional neuroendocrine and other responses to this agent.

Publications:

Taylor, P.A., Garrick, N.A., Tamarkin, L., Murphy, D.L., and Markey, S.P.: Diurnal rhythms of N-acetylserotonin and serotonin in cerebrospinal fluid of monkeys. Science 228: 900, 1985.

Ziegler, M.G., Kennedy, B., Holland, O.B., Murphy, D., and Lake, C.R.: The effects of dopamine agonists on human cardiovascular and sympathetic nervous systems. Int. J. Clin. Pharmacol. Ther. Toxicol. 23: 175-179, 1985.

Mueller, E.A., Murphy, D.L., and Sunderland, T.: Neuroendocrine effects of m-chlorophenylpiperazine (m-CPP), a serotonin agonist, in humans. J. Clin. Endocrinol. Met., in press.

Zamir, N., Skofitsch, G., Eskay, R., and Jacobowitz, D.M.: Distribution of immunoreactive atrial natriuretic peptide in the central nervous system of the rat. Brain Res., in press.

Zamir, N.: On the origin of Leu-enkephalin and Met-enkephalin in the rat neurohypophysis. Endocrinology, in press.

Zamir, N., Quirion, R., and Segal, M.: Distribution of immunoreactive prodynorphin and proenkephalin derived peptides and opiate receptors in the rat hippocampus. Neuroscience, in press.

Zamir, N., Weber, E., Palkovits, M., and Brownstein, M.J.: Distribution of immunoreactive metorphamide (adrenorphin) in discrete nuclei of the rat brain; comparison with met-enkephalin-Arg-Gly-Leu. Brain Res., in press.

Zamir, N., Skofitsch, G., and Jacobowitz, D.M.: Distribution of immunoreactive-melanin concentrating hormone in the central nervous system of the rat. Brain Res., in press.

Zamir, N. and Maixner, W.: Interactions between cardiovascular and pain regulatory systems. Ann. N.Y. Acad. Sci., in press.

Zamir, N., Skofitsch, G., Bannon, M.J., and Jacobowitz, D.M.: Melanin-concentrating hormone: A novel peptide neuronal system in the rat brain and pituitary gland. Proc. Natl. Acad. Sci. USA, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00338-05 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Families of Origin in Obsessive-Compulsive Illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carol F. Hoover D.S.W. LCS NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was discontinued following the departure of the principal investigator from NIMH in July, 1984.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00339-04 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropharmacology of Cognition and Mood in Geriatric Neuropsychiatry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Trey Sunderland, Staff Physician LCS NIMH

COOPERATING UNITS *(if any)*

LCM, NIMH; NIDA;
Enzor Research Foundation

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.0	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous work from the Neuropharmacology of Cognition project has addressed the close association between attention and motivational state of depressed patients and normals with their cognitive performance. Testing with intravenous naloxone has demonstrated that behavioral changes such as increased depression and anxiety can lead to impaired memory performance. Those observations have led us to expansion in our investigations examining the effects of neuropharmacolgic manipulations on the affective and cognitive responses of Alzheimer's patients. This year, we have observed that these patients show marked cognitive and affective sensitivity to the cholinergic antagonist scopolamine and that the behavioral response of Alzheimer patients to naloxone is at a lower dose than previously found with young normals. We are currently examining the effect of age on the response to scopolamine and naloxone with a cohort of aged controls. We are also now in the initial stages of testing the cognitive, behavioral and neuroendocrine reactions of Alzheimer patients and controls to the cholinergic agonists, arecholine and nicotine. In our continued efforts to explore the links between cognition and affective state and its neuropharmacology, we are also actively investigating a therapeutic drug strategy with monoamine oxidase inhibitors in this population of dementia patients.

PHS 6040 (Rev. 1/84) GPO 914-918

Other collaborative professional personnel engaged on the project:

D. L. Murphy	Chief	LCS NIMH
H. Weingartner, Ph.D.	Unit Chief	LPP NIMH
P.N. Tariot, M.D.	Staff Physician	LCS NIMH
P. Newhouse, M.D.	Staff Physician	LCS NIMH
R. M. Cohen, M.D.,Ph.D.	Section Chief	LCM NIMH
M. Gross, M.D.	Staff Physician	LCM NIMH
E.A. Mueller, M.D.	Staff Physician	LCS NIMH

Project Description:

Objectives: Cognitive impairments and mood have been linked to a number of neurotransmitter pathways on the basis of studies in various neuropsychiatric illnesses and pharmacologic challenges in normals. Specifically in Alzheimer's disease, deficits in the cholinergic system have been implicated etiologically, and numerous attempts at pharmacologic cholinergic replacement therapy have been made. To help elucidate the cholinergic hypothesis of Alzheimer's disease, cholinergic agonist and antagonist agents are given to Alzheimer patients, normal controls and in some cases, elderly depressives. Differences between groups in cognitive and behavioral measures following cholinergic and other agents should help in the understanding of their interrelationships as well as the possible differences in sensitivity across groups, a finding which may have direct therapeutic and diagnostic implications.

Methods Employed:

Behavioral and Psychological Assessment: The diagnosis of Alzheimer disease is based on the criteria of the DSM-III as well as the Dementia Rating Scale of Hughes and coworkers in St. Louis. The latter scale is an amalgam of multiple scales including the Blood Dementia Scale, the Face-Hand test, the Pfeiffer short portable mental status questionnaire and others and provides a measure of severity of illness. The clinical diagnosis is made only after thorough evaluation with the exclusion of any patients suspected of having multi-infarct or other forms of non-Alzheimer dementia. The diagnosis of major affective disorder in the elderly is made on the basis of DSM-III criteria.

Mood and other behavioral characteristics are measured with global (15-point) rating scales, the Brief Psychiatric Rating Scale and the Hamilton depression rating scale where appropriate. A new dementia mood assessment scale has been developed specifically for this population because of the difficulty found using self-rating forms or other mood scales designed for general depressed patients. Activities of daily living are also assessed by family and staff throughout the hospitalization with a measurement tool developed as part of this project.

For the evaluation of cognitive skills, a large number of tests are employed. There are several routine psychometric measures including the Wechsler memory quotient in addition to a series of recently designed or modified tasks for this population. These tests assess effortful and semantic memory and include measures of attention, free recall and recognition memory. Though primarily evaluating verbal memory, some of the tests do measure visual memory and sustained motor attention.

Biological Assessment: Plasma, platelets, urine and cerebrospinal fluid are collected for measurement of enzymes, hormones, levels of biogenic amines and their metabolites. The dexamethasone suppression test and the TRH stimulation tests are also used. Some patients and controls are asked to undergo a skin biopsy for culturing and subsequent biochemical testing of the skin fibroblasts.

Major Findings:

Continuing the study of the opiate system in humans, a series of doses of naloxone have been administered to a series of 12 Alzheimer patients by Dr. Pierre Tariot. Significant behavioral activation, restlessness and irritability have been found even at 100 mg/kg, a dose 20-60 fold lower than that required for such changes in young normals. The patients have also demonstrated increased irrelevant verbal associations in memory tasks at this dose with no suggestion of memory improvement at this dose or doses as high as 2 mg/kg. Dr. Michael Gross and Dr. Pierre Tariot are currently testing a series of elderly controls to find whether this apparent increased sensitivity is age related.

Within the cholinergic systems, we have recently completed work on the initial stages of dose-response scopolamine testing in the Alzheimer patients. The dementia patients showed a marked behavioral and cognitive sensitivity to anticholinergic blockade. Patients show significantly increased restlessness, irritability and agitation on intravenous doses of scopolamine as low as 0.25 mg. At this same dose, they also display marked impairments of their cognitive performances in many areas, most notably in processes which require semantic memory. This sensitivity is found at doses which are much less than those previously required for cognitive effects in young normals. Older controls are now being tested in the exact same paradigm.

Significance to Biomedical Research and the Program of the Institute:

Despite many neuropathologic clues to the underlying etiology of Alzheimer's disease, there remains no recommended treatment course. The cognitive impairments continue to progress and behavior disturbances loom large as families and medical institutions struggle to cope with increasing burdens of care. In our study of two brain neurotransmitter systems, the opiate and cholinergic, we have noted a strong correlation between the intensity of mood disturbance in the dementia population and the degree of cognitive impairment. This is not so surprising given the previous findings of a close relationship between the performance of depressed patients on motor and cognitive tasks and their depression. That these deficits are reversible with appropriate treatment of the underlying depression suggests strongly that at least some of the cognitive impairments in dementia patients could be related to mood disturbances. It may be that deficits of cognition and behavioral abnormalities are more closely tied centrally and thus amenable to pharmacologic treatment.

The methods of evaluating behavior and mood in the Alzheimer population are more complex than with depressed patients. The difficulties in communication and general lack of accurate self-reporting pose problems for measuring the interplay of cognition and emotion in dementia, but more objective tools are being developed which should help in this process. Once in place, pharmacologic manipulations can be more carefully analyzed for both cognitive and vehavioral effects and interactions. In addition to showing that behavior and cognition are linked more closely than

expected, the work with naloxone and scopolamine may also be demonstrating that the dementia patients are quite sensitive to these drugs. While diagnostic and staging implications for these differences in drug sensitivity may exist, it is more likely that these studies will help us reevaluate previous experience with cholinergic and other therapeutic agents in this population and help plan future studies. Our own studies will be focusing on the effects of antidepressant treatment on the mood and cognitive functioning of dementia patients.

Proposed Course:

We have already admitted and intensely studied over 50 Alzheimer patients on our inpatient unit and evaluated dozens more as outpatients. While continuing our initial neuropharmacologic challenge studies with naloxone and scopolamine in elderly controls and other populations, we have begun to study the effects of two cholinergic agonists, arecholine and nicotine, in both dementia patients and controls. In addition, we are continuing our study of low and middle-doses of the selective monoamine oxidase-B inhibitor, deprenyl. Now that we have several instruments designed to measure mood changes in this population, the analysis of results with deprenyl and future drugs should be more reliable. Correlation of various neurochemical measures with cognitive and behavioral results will also be possible. Of particular interest and immediately available to us is the return of former patients. Longitudinal cognitive, behavioral and neurochemical data could be invaluable in the study of illness progression. Finally, we have already begun and will continue to evaluate a number of elderly depressed patients in the hospital. Their baseline evaluations and treatment responses could prove helpful for comparison purposes both with the dementia patients and the elderly controls.

Publications:

Grimes, A.M., Grady, C.L., Foster, N.L., Sunderland, T. and Patronas, N.J.: Central auditory function in Alzheimer's disease. Neurology 35:352-358, 1985.

Tariot, P.N., Sunderland, T., Murphy, D.L., Cohen, R.M., Weingartner, H. and Makohon, R.: How memory fails: A theoretical model. Geriatric Nursing 6:144-147, 1985.

Sunderland, T., Tariot, P.N., Murphy, D.L., Weingartner, H., Mueller, E.A. and Cohen, R.M. Scopolamine challenges in Alzheimer's Disease. Psychopharmacology, in press.

Tariot, P.N., Sunderland, T., Weingartner, H., Murphy, D.L., Cohen, M.R, and Cohen, R.M. Low and high dose naloxone in dementia of the Alzheimer type. Psychopharmacol. Bull., in press.

Sunderland, T., Tariot, P.N., Murphy, D.L., Weingartner, H., Mueller, E.A. and Cohen, R.M. Cognitive and behavioral sensitivity to scopolamine in Alzheimer patients and controls. Psychopharmacol. Bull., in press.

Sunderland, T., Tariot, P., Mueller, E.A., Newhouse, P., Murphy, D.L. and Cohen, R.M. TRH stimulation test in dementia of the Alzheimer type and elderly controls. Psychiatry Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02218-02 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biophysical Approaches to Medical Therapeutics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jonathan L. Costa, Staff Physician LCS NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was discontinued following the departure of the principal investigator from NIMH in July, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00425-09 LCS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Peripheral and Central Catecholamines in Hypertension and Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Juan M. Saavedra, M.D. Chief, Unit on Preclinical Neuropharmacology
Section on Clinical Pharmacology LCS, NIMH

Others: See Attached Sheet

COOPERATING UNITS *(if any)*

Department of Neurology, Cornell University, New York; INSERM, France; NIGMS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We studied the role of peripheral and central catecholamines and angiotensin II in the regulation of cardiovascular function.

We established that specific brain areas containing adrenaline neurons control both catecholamine and vasopressin release and influence blood pressure and heart rate.

We determined the precise source of intermediate and posterior lobe pituitary catecholamines.

There are close interactions between β-adrenergic receptors and arterial angiotensin-converting enzyme.

The brain angiotensin system, both at the converting enzyme and at the receptor levels, is involved in cardiovascular control in the spontaneously hypertensive rat.

PHS 6040 (Rev. 1/84) GPO 914-918

Other Professional Personnel:

Laura M. Plunkett	Staff Fellow	PRAT Program, NIGMS
Masami Niwa	Visiting Fellow	LCS, NIMH
Anita Israel	Guest Researcher	LCS, NIMH
Fernando Correa	Guest Researcher	LCS, NIMH
Markku Koulu	Visiting Fellow	LCS, NIMH
Claude Chevillard	Established Investigator	INSERM, Montpellier, France
Donald Reis	Professor of Neurology	Cornell University

Project Description:

Objectives:

To study the role of central and peripheral catecholamines, other biogenic amines and prostaglandins in hypertension and stress. This project has been extended to include the study of neuropeptides, notably angiotensin II, in the regulation of cardiovascular function.

Methods Employed:

Neuroanatomical, surgical, biochemical (HPLC, RIA, radioenzymatic), and autoradiography with image analysis coupled to computerized microdensitometry.

Major Findings:

The C_1 area of the brainstem containing adrenaline neurons influences arterial pressure and heart rate by its regulation of catecholamine and vasopressin secretion.

The intermediate and posterior lobes of the pituitary gland have a catecholamine innervation originated mainly in the brain. Part of the norepinephrine in the posterior lobe originates in the superior cervical ganglia.

The β-adrenergic blocking agent pindolol decreases plasma and arterial angiotensin-converting enzyme activity in young, spontaneously hypertensive rats.

Angiotensin-converting enzyme is reduced in discrete brain areas of spontaneously hypertensive rats.

Increased affinity for angiotensin II receptors is present in the nucleus of the solitary tract of spontaneously hypertensive rats.

Significance to Biomedical Research:

We have demonstrated that a specific area of the brain, containing adrenaline neurons, can control cardiovascular function through a modulation

of both catecholamine and vasopressin release. These observations support the hypothesis advanced earlier of a role for central adrenaline neurons in blood pressure regulation.

The precise analysis of the origin of the posterior and intermediate pituitary lobe catecholamines has established that the posterior lobe function could be subject to a dual sympathetic control, both central and peripheral through the superior cervical ganglia.

Peripheral β-adrenergic receptors may play a role in the local regulation of angiotensin II formation, mainly at the mesenteric artery level. This observation suggests an intimate relation between the sympathetic and the angiotensin systems in blood pressure control.

A role for the participation of the brain angiotensin system in blood pressure regulation is supported by two independent findings: angiotensin-converting enzyme and angiotensin II receptors are altered in specific brain areas of spontaneously hypertensive rats.

It is hoped that these studies will help to clarify the role of the sympathetic and the angiotensin systems in cardiovascular regulation.

Proposed Course of Project:

We plan to utilize the newly developed autoradiographic techniques to study the role of angiotensin II, α and β-adrenergic receptors in the central regulation of cardiovascular function and stress, and to focus in the central and peripheral interactions between these two systems.

Publications:

Chevillard, C., Niwa, M. and Saavedra, J.M.: Angiotensin-converting enzyme in discrete forebrain areas of spontaneously hypertensive rats. Brain Res. 309: 389-392, 1984.

Niwa, M. Israel, A. and Saavedra, J.M.: Pindolol decreases plasma angiotensin converting enzyme activity in young spontaneously hypertensive rats. Clinical and Experimental Hypertension. Theory and Practice, A6, 1765-1768, 1984.

Niwa, M., Israel, A. and Saavedra, J.M.: Pindolol decreases plasma angiotensin converting enzyme activity in young spontaneously hypertensive rats. Eur. J. Pharmacol. 110: 133-136, 1985.

Ross, C.A., Ruggiero, D.A., Park, D.H., Joh, T.H., Sved, A.F., Fernandez-Pardal, J., Saavedra, J.M. and Reis, D.: Tonic vasomotor control by the rostral ventrolateral medulla; effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial pressure, heart rate, and plasma catecholamines and vasopressin. J. Neuroscience 4: 474-494, 1984.

Saavedra, J.M.: Radioenzymatic assay of biogenic amines. In S. Parvez, T. Nagatsu, I. Nagatsu and H. Parvez, (Eds.): Methods in Biogenic Amine Research, Elsevier Science Publishers B.V., 1983, pp. 257-283.

Saavedra, J.M.: The use of enzymatic radioisotopic microassays for the quantification of β-phenylethylamine, phenylethanolamine, tyramine and octopamine. In A.A. Boulton, G.B. Baker, W.G. Dewhurst, and M. Sandler (Eds.): Neurobiology of the Trace Amines, The Humana Press, 1984, pp. 41-55.

Saavedra, J.M.: Central and peripheral catecholamine innervation of the rat intermediate and posterior pituitary lobes. Neuroendocrinology 40: 281-284, 1985.

Saavedra, J.M., Fernandez-Pardal, J., Torda, T., Reis, D. and Ross, C.: Dissociation between rat hypothalamic and brain stem PNMT after stress and between hypothalamic catecholamines and PNMT after midbrain hemi-transections. In E. Usdin and R. Kvetnansky (Eds.): Catecholamines and Other Transmitters in Stress, Stress: role of catecholamines and other neurotransmitters, Bell and Bain, Ltd. Glasgow; 1984, pp. 134-145.

Correa, F.M.A. and Saavedra, J.M.: Isotopic-enzymatic microassay of histamine at low femtomole range in microgram amounts of brain tissue. Brazilian J. Med. Biol. Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00428-06 LCS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Carboxyl Methylation: A Post Translational Modifier of Protein Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Juan M. Saavedra, M.D. Chief, Unit on Preclinical Neuropharmacology
Section on Clinical Pharmacology LCS, NIMH

Others: None

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is a correlation between the exocytotic process and protein carboxyl methylation. In the adrenal medulla, repeated immobilization stress results in a progressive decrease of endogenous methyl acceptor proteins. In the posterior gland, there is an association between hormone release and methylation of endogenous substrate proteins.

PHS 6040 (Rev. 1/84) GPO 914-916

Project Description:

Objectives:

To study the relationship between enzymatic carboxyl methylation and exocytotic release in pituitary gland and brain nuclei, and to characterize newly detected endogenous methyl acceptor proteins in pituitary gland.

Methods Employed:

Enzymatic, pharmacologic, gel electrophoresis, HPLC, RIA.

Major Findings:

There is a gradual decrease in the amount of methyl acceptor proteins in the rat adrenal medulla when submitted to repeated immobilization stress. Increased hormone release from isolated posterior pituitaries is associated with increased protein carboxyl methylation in this organ.

Significance to Biomedical Research:

We have found evidence of a correlation between exocytotic processes and protein carboxyl methylation. Carboxyl methylation, by regulating the exocytotic process, may be an important factor in hormone release.

Proposed Course of Project:

We will attempt to characterize the specific endogenous proteins which are substrates for the carboxyl methylation process in both the pituitary and the adrenal medulla.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00433-05 LCS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Neuropeptides in Neuroendocrine Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Juan M. Saavedra, M.D. Chief, Unit on Preclinical Neuropharmacology
Section on Clinical Pharmacology LCS, NIMH

Others: See Attached Sheet

COOPERATING UNITS *(if any)*

LCM, NIMH; Unit on Radioimmunoassay, Institute Pasteur, Paris, France; NIGMS; HE, NHLBI, University of Heidelberg, West Germany

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project studies the interrelationships between several neuropeptides (angiotensin II, vasopressin, corticotropin releasing factor, somatostatin and substance) and their role in neuroendocrine regulation.

We developed new quantitative autoradiographic methods with computerized microdensitometry to study receptors for neuropeptides and enzymes involved in neuropeptide metabolism in discrete nuclei of the brain and peripheral organs of the rat.

We have found angiotensin II, somatostatin, substance P, atrial natriuretic factor, and high affinity receptors localized in specific areas of the rat brain.

Alterations in fluid metabolism increases the number of angiotensin receptors in selective brain areas and in the pituitary gland.

Angiotensin-converting enzyme (Kininase II) was also quantitated by autoradiography in selected rat brain nuclei. This enzyme appears to be low in the cerebrospinal fluid of schizophrenics.

PHS 6040 (Rev. 1/84) GPO 914-918

Other Professional Personnel:

Laura M. Plunkett	Staff Fellow	PRAT Program, NIGMS
Masami Niwa	Visiting Fellow	LCS, NIMH
Anita Israel	Guest Researcher	LCS, NIMH
Fernando Correa	Guest Researcher	LCS, NIMH
Kyriaki Gerozissis	Research Associate	Pasteur Institute Paris, France
Catherine Rougeot	Chemist	" "
Fernand Dray	Head, Unit on Radioimmunoassay	" "
Massako Kadekaro	Visiting Scientist	LCM, NIMH
Louis Sokoloff	Chief	LCM, NIMH
Paul Gross	Visiting Fellow	LCM, NIMH
H. Beckmann	Professor of Psychiatry	Univ. of Heidelberg, West Germany
Richard McCarty	Guest Researcher	IR HE, NHLBI

Project Description:

Objectives:

To study the functions and interactions of angiotensin II, vasopressin, corticotropin releasing factors, somatostatin, and substance P in brain nuclei, pituitary gland and other peripheral organs, and their role in neuroendocrine regulation.

Methods Employed:

Neuroanatomical, biochemical, RIA, HPLC, autoradiography with image analysis, and computerized microdensitometry.

Major Findings:

We have developed quantitative autoradiographic methods with image analysis coupled to computerized microdensitometry to localize and quantitate receptors for neuropeptides in discrete brain nuclei. Similar techniques have been applied to the study of angiotensin-converting enzyme (kininase II). Complete kinetic analysis of receptors and enzymes can be performed in single rat brains.

We have localized and quantitated receptors for angiotensin II, somatostatin, substance P and atrial natriuretic factor in discrete rat brain nuclei, individual rat sympathetic ganglia, pituitary and adrenal glands, and kidney.

Angiotensin II receptors in brain nuclei and pituitary gland are regulated by alterations in fluid metabolism.

Significance to Biomedical Research:

These studies will help to clarify the role of neuropeptides such as

angiotensin II, vasopressin, corticotropin-releasing factor, somatostatin, and substance P in neuroendocrine regulation.

Proposed Course of Project:

We plan to study further the interactions between those neuropeptides, with particular emphasis in the regulation of neuropeptide receptors and peptidases in discrete brain areas.

Publications:

Beckmann, H., Saavedra, J.M., and Gattaz, W.F.: Low angiotensin converting enzyme activity (Kininase II) in cerebrospinal fluid of schizophrenics. Biological Psychiatry 10: 679-684, 1984.

Chevillard, C. et Saavedra, J.M.: L'angiotensine 1 - convertase: modification de l'activite dans le SNC du rat depourvu de vasopressine. Act. Pharm. Biol. Clin. 2: 171-174, 1983.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00447-16 LCS

PERIOD COVERED

October 1, 1984, through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Amine neurotransmitters and metabolites in mental illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

William Z. Potter, M.D., Ph.D., Chief, Section on Clinical Pharmacology, Laboratory of Clinical Science, NIMH

COOPERATING UNITS *(if any)*

Clinical Psychobiology Branch; Neuroscience Branch; Child Psychiatry Branch, NIMH; and Laboratory of Clinical Studies, NIAAA

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Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	2.5	1.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alterations of amine neurotransmitter systems (norepinephrine (NE), serotonin (5HT) and dopamine (DA)) have been indirectly implicated in the pathophysiology of the major mental illnesses, depression and schizophrenia. We have applied new techniques to study cerebrospinal fluid (CSF), plasma and urine from drug-free patients with affective illness and schizophrenia using more sensitive and comprehensive characterization of the neurotransmitter systems as well as selective measures of post-synaptic function. New findings include the following:

1. Bipolar (BP) and unipolar (UP) depressed patients now appear to be distinguishable by both presynaptic measures in CSF, plasma and urine (lower levels of NE and/or its metabolite MHPG in BPs) and a post-synaptic measure in blood (higher affinity of beta lymphocyte receptors in BPs, see below).
2. Beta adrenergic receptors in lymphocytes have been characterized in BP, UP, bulemic and anorexic patients as well as controls. The ratio of affinities for two states of the receptor in general parallel plasma NE (not epinephrine) yet can still distinguish groups from one another even when plasma NE does not.
3. Biochemical evidence in man using 5HT and DA metabolites in CSF and in animals using parent amines as well as metabolites points to functional control of DA turnover by 5HT, at least under certain conditions. This has important implications for understanding the role of 5HT function in psychiatric illness.
4. When activity and diet are controlled, the apparent circadian rhythm in plasma MHPG disappears and a robust one in plasma HVA emerges.

Other Professional Personnel:

Matthew Rudorfer	Senior Staff Fellow	LCS/NIMH
Robert Golden	Medical Staff Fellow	LCS/NIMH
Michael Sherer	Medical Staff Fellow	LCS/NIMH
Hans Agren	Visiting Fellow	LCS/NIMH
Neil Buckholtz	Guest Researcher	IPA/NIMH
Markku Linnoila	Chief	LCS/NIAAA
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David Pickar	Chief, Section on Clinical Studies	NSB/NIMH
Judith Rapoport	Chief, Child Psychiatry Branch	CHP/NIMH

Project Description:

The characterization of the functional state of three amine neurotransmitter (NT) systems, norepinephrine (NE), serotonin (5HT) and dopamine (DA), in depression and other major psychiatric illnesses such as schizophrenia continues to be a major ongoing project. We are adding to that the characterization of the epinephrine (EPI) system. Over a decade of method development and clinical studies has led to the identification of numerous sources of variance which we have only recently been able to control. In many instances, it remains an open question whether appropriate controls are possible.

Simultaneous studies, particularly in depressive illness of neuroendocrine responsivity, peptidergic systems and post-synaptic receptors complement rather than supplant studies of the neurotransmitters themselves and still tend to be conceptualized as either dependent on or co-variants of NT function. Even DST escape can be viewed as an HPA abnormality reflecting regulation of the adrenergic/noradrenergic system(s).

Continued expansion of our understanding of the regulation of these NT systems in both healthy volunteers and psychiatric patients seems certain to at least provide tools to subtyping psychiatric illness and predicting response to treatment. Following this classic approach with new techniques can fulfill the more fundamental goal of identifying the pathophysiology of at least some mental illness.

Methods:

Because of the advances in analytical techniques, a central laboratory has been organized and is described in a separate project summary. Thus, our current biochemical techniques are not described here.

Selection of subjects paying particular attention to such issues as age of onset, frequency of recurrence of episodes, and family history is given great emphasis. Whenever feasible, extended (over 1 month) drug-free periods are required before biological samples are obtained--a 3-week period is our current minimum although new data suggests that even this length may be inadequate if a patient has been on tricyclic antidepressants and is definitely inadequate if they have received chronic neuroleptics or monoamine oxidase inhibitors.

Patients are also characterized according to length on a low monoamine diet as well as number of days in hospital. This latter parameter is of particular interest since many depressed patients are studied after brief (sometimes only overnight) hospitalization and then transferred to outpatient status. With the expansion of outpatient studies, some procedures are performed in some studies without hospitalization. The consequences of this alternate methodology are just beginning to be appreciated.

"Control" subjects must be drawn from both hospitalized and "outpatient" age- and sex-matched individuals who are asked to be on diet. It appears that for comparisons of urine and CSF hospitalization can be a critical variable. Therefore, a comparison of "controls" under different conditions has become an essential component of our design.

Major Findings:

1. We continue to find that unipolar (UP) and bipolar (BP) depressed patients can be distinguished with plasma and CSF measures of NE and its metabolites as well as in urine. Analysis of a large Scandinavian sample by Dr. Agren reveals for the first time that when sources of variance are adequately controlled MHPG in the CSF is lower in BP than UP patients. Moreover, even in subgroups of patients in whom direct NE measures overlap, the affinity state of beta lymphocyte receptors provides a measure consistent with lower basal NE in BP patients. These findings support the possibility that a battery of biological tests to identify BP vs UP patients may be feasible.

2. Measurement of beta lymphocyte high and low affinity sites is consistent with other subgroups of patients that have altered noradrenergic function. For instance, in collaboration with the Section on Biomedical Psychiatry, we find a shift toward a high affinity state in bulimic patients who have consistently low resting levels of plasma NE. Interestingly, despite the characterization of lymphocyte beta receptors as preferential type 2 with selectivity for EPI, circulating levels of this neurohormone are not related to beta lymphocyte state whereas levels of NE are. This appears to be true across both depressed patients, those with eating disorders and control subjects further supporting the central role of NE dysfunction in the specified psychiatric disorders.

3. The well-recognized but unexplained covariance of the 5HT and DA metabolites, 5HIAA and HVA respectively, in CSF now appear to reflect a functional interaction whereby under basal conditions, and in selected brain regions, 5HT exerts a marked effect on DA turnover. This conclusion arises from statistical modeling of large but independent samples of human CSF as well as investigation of multiple discrete brain areas in the rat and dog. Since such an interpretation is consistent with neurophysiological, behavioral and neuroanatomical studies, we suggest that apparent abnormalities of the 5HT system must be viewed in the context of the status of the DA system. Thus, in a recent analysis of data from a study attempting to replicate low 5HIAA in the CSF as a correlate of suicidality, we see low HVA as the clearly significant factor.

4. Collaborative studies with the CPB and NSB confirm that normal healthy male volunteers have a marked endogenous circadian pattern of plasma HVA excretion characterized by a peak in the middle of the night and episodic daytime rises abolished by controlling activity and time of eating. In contrast, after the preceding controlled conditions, no significant rhythms in plasma MHPG could be detected, suggesting that the variable late afternoon/early evening "peak" previously reported by others reflects the varying degrees of influence of exogenous environmental variables on sympathetic nervous system outflow which is expected to be higher during normal daytime hours.

Significance to Biomedical Research and to the Program of the Institute:

The major theories about the biological causes of the most prevalent severe psychiatric disorders, depression and schizophrenia, center on monoamine neurotransmitter systems. This project applies sophisticated laboratory assays directly to human studies of monoamine metabolism. Results expand our understanding of the role of norepinephrine and other neurotransmitters mainly in depression. The personal and social costs of this illness are great. Insofar as careful clinical research, drawing on basic biochemical techniques, can identify biological factors in these disorders, specific pharmacologic treatments can be developed and tested in therapeutic trials.

Proposed Course:

We will test the repeated finding of decreased NE output in bipolar depressed patients against other physiologic indices, in particular urinary hydroxy-melatonin output and beta lymphocyte receptor measures to see if true, selective biochemical measures of bipolar illness are possible.

We will continue to evaluate what are the "controls" and work out determinants other than production and release of the plasma concentration of transmitters and their metabolites--such as renal clearance. Furthermore, we shall design additional studies to characterize the conversion rates of NE to various metabolites in specific normetanephrine so as to interpret new findings.

We will continue to collaboratively study schizophrenic as well as any of our own depressed patients who have concomitant psychosis focusing on the DA system and its interaction with NE. A major focus will be on the possible role of increased plasma HVA as a correlate of psychosis and of patterns of HVA as a circadian marker.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01850-08 LCS

PERIOD COVERED
October 1, 1984, through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical pharmacology of antidepressants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

William Z. Potter, M.D., Ph.D., Chief, Section on Clinical Pharmacology, Laboratory of Clinical Science, NIMH

COOPERATING UNITS *(if any)*
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.25	3.5	1.75

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antidepressant medications are prescribed to millions of Americans, many of whom receive the drug for months to years. Despite increasingly sophisticated studies in animals and the development of more biochemically specific antidepressants, the therapeutic mechanism of action in man remains unknown. Comparison of effects on specific neurotransmitters and their metabolites in cerebrospinal fluid (CSF), plasma and urine in the same patients continues to expand and is complemented by physiologic, behavioral and neuroendocrine measures, allowing for clearer systems interpretations of changes. Findings of particular interest include the following:

1. We have now found that all chemically distinct antidepressant treatments which we have studied (seven to date) share the property of decreasing norepinephrine (NE) turnover; these include the "atypical" drugs bupropion and citalopram and, in initial studies, alprazolam.
2. Non-response after the atypical new putative antidepressant bupropion is associated with elevated plasma homovanillic acid, the major dopamine (DA) metabolite, and may be explained by the accumulation of high concentrations of pharmacologically active metabolites in plasma and CSF.
3. Monoamine oxidase inhibitors which decrease plasma NE as well as agents which increase it produce elevated urinary hydroxymelatonin, showing that despite decreased turnover antidepressants maintain or enhance NE function.
4. An apparently selective probe of serotonin (5HT) function and hence drug effects on that system has been developed by using intravenous administration of a 5HT uptake inhibitor at doses that do not cause nausea.

PHS 6040 (Rev. 1/84) GPO 904-917

Other Professional Personnel:

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Dennis L. Murphy	Chief	LCS/NIMH
Herbert Weingartner	Psychologist	LPP/NIMH

Project Description:

Our central aim is to understand the effects of major somatic antidepressant treatments on the monoamine neurotransmitter systems in man. Systematic studies of drug action in normal volunteer controls and depressed patients controlling for pharmacokinetic and pre-drug physiologic variance has permitted demonstration of both predicted and unexpected biochemical alterations following treatment with drugs having widely differing acute primary effects.

Comparison of biochemical effects in CSF, plasma and urine in the same patients is now feasible with new, efficient high performance liquid chromatography assays, and, when coupled with physiologic, behavioral and neuroendocrine measures, allows for clearer systems interpretations of changes. State-of-the-art measures of norepinephrine (NE), serotonin (5HT), dopamine (DA) and their metabolites are made under controlled conditions both cross sectionally in time and longitudinally in order to identify interrelationships, to test assumptions about the regulation of these neurotransmitter systems, and therefore to definitively describe effects of antidepressants as they relate to these neurotransmitter systems.

Methods:

The neurotransmitter systems of patients with either unipolar or bipolar major affective disorder are characterized after at least a 3-week drug-free period which we are attempting to extend further and then between the 3rd and 5th week following antidepressant treatment. Certain parameters, such as urinary transmitter and metabolite concentrations, are studied repeatedly following the beginning of each treatment. Parallel studies are performed in healthy volunteers when feasible as described below.

Treatments are ideally administered so as to produce maximal effects on the presumed target biochemical system such as inhibition of NE uptake after desipramine (DMI), of 5HT uptake after citalopram or fluvoxamine, and of MAO-Type A after clorgyline using control of pharmacokinetic variance (blood levels of DMI, citalopram or bupropion) or biochemical indices (MHPG decrease after clorgyline). In the case of lithium and ECT, standard regimens are followed.

Novel antidepressants with no clear biochemical specificity such as bupropion, S-adenosylmethionine, and alprazolam are also studied.

Studies in college age volunteers housed on the unit are of shorter duration (up to two weeks of active drug) and include DMI, S-adenosylmethionine and lithium.

Specialized pharmacokinetic and baseline biochemical studies are performed in volunteers age- and sex-matched to our accumulated patient population. These volunteers come to the clinic on the day of the study or are admitted for an overnight accommodation to the research unit.

Analysis of NE, 5HT, DA and their metabolites is carried out as described in a separate report, Z01 MH 01855-01 LCS. Urinary hydroxy-melatonin is currently measured by GC-MS in the laboratory of Dr. Markey. Hormone concentrations are measured by RIA either in collaboration with other groups or by Hazleton Laboratories.

Findings to Date:

1. Consistent with animal studies and our previous reports, we show selective decreases of norepinephrine (NE) turnover after an increasingly wide range of antidepressant treatments. Both bupropion, with unknown primary effects, and citalopram, a new 5HT uptake inhibitor, have been found to have such an effect. The specific pattern of change reflected in plasma NE varies with each treatment but supports our notion that the overall efficiency of the noradrenergic system is increased following antidepressants.

2. Urinary hydroxy-melatonin, an integrated measure of melatonin release which is primarily under noradrenergic control, is increased rather than decreased after antidepressants, including monoamine oxidase inhibitors (MAOIs). Since MAOIs reduce plasma NE as well as total NE turnover, these findings demonstrate increased NE function in vivo in humans despite reduced NE output.

3. Preliminary evidence on lymphocyte beta adrenergic receptors before and after antidepressant treatments provides additional characterization of the NE system. The major treatment-associated alterations appear to be on variability of the ratio of high and low affinity states (which is the best known correlate of function) rather than their absolute level. Treatment therefore produces a more regulated NE system in terms of receptor sensitivity as well as in terms of turnover.

4. We have begun to administer electroconvulsive therapy (ECT) as part of a formal protocol. To date, four depressed patients have been able to fully participate in studies. Preliminary results show that an initially exaggerated response of plasma norepinephrine to an orthostatic challenge diminishes during the course of ECT, although this did not occur in a patient who did not respond to the treatment. Furthermore, we have observed, in two of three patients, an increase in the cerebrospinal fluid concentration of the major metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) following a course of ECT. Thus, as expected for as nonspecific a treatment as ECT, multiple transmitter systems may be involved in its mechanism of action.

5. Both treatment non-response and the unexpected elevation of plasma HVA (the dopamine metabolite) seen in some patients after bupropion which we reported last year are now found to be associated with high concentrations of the drug's metabolites in plasma and cerebrospinal fluid. We therefore have presumptive evidence that there are biologically active metabolites of bupropion which have a different spectrum of action than the parent compound and which may explain the drug's multiple and variable effects on neurotransmitters. Interestingly, bupropion is the only antidepressant drug which we have investigated which does not reduce the 5HT metabolite, 5HIAA, in CSF although it does reduce the NE metabolite, MHPG.

6. Using a rat model for 5HT uptake inhibitor-induced stimulation of plasma prolactin, we find that the response is a function of rate of administration as well as dose with rapid development of tachyphylaxis to subsequent doses. Furthermore, changes in DA turnover precede effects on 5HT turnover, suggesting that rapid increases of intrasynaptic 5HT in these projections which impinge on DA neurons may provide the primary mechanism whereby 5HT uptake inhibition transiently stimulates prolactin release.

7. In keeping with the preclinical findings described above, we administered intravenous chlorimipramine (a 5HT uptake inhibitor) to volunteers and patients, producing an immediate prolactin rise without increasing growth hormone at doses which did not produce nausea. This technique may provide a more selective probe of 5HT-influenced neurohormone regulation.

Significance to Biomedical Research and to the Program of the Institute:

Understanding of the mechanism(s) of action of antidepressant treatments produces improved therapeutics, new drugs, tools for studying and investigating the underlying pathophysiology of depression and therefore, ultimately, provides the basis for prevention.

From a therapeutic point of view pharmacokinetic studies have been critical to removing problems related to inappropriate dosing. Moreover, the systematic study of biochemically selective (clorgyline) and novel presumably less toxic agents (SAMe, bupropion) provide treatments which are effective in many patients who do not respond to standard antidepressants.

Of ultimate importance is the continued finding that changes of the noradrenergic system are always involved in the action of somatic antidepressant treatments. Although simple deficit or excess catecholamine hypotheses of depression do not explain drug action, it seems clear that to understand the mechanism we must understand the role of NE. More and more investigators are "returning" to studies of the NE system in man.

Proposed Course:

1. Complementary studies with newly available selective NE and 5HT uptake inhibitors are planned to test the generalizability of our findings. Since both clinical and preclinical studies continue to identify effects on the NE system resulting from all antidepressant drugs, including new ones as described above,

we plan to turn our attention to more specific questions. For instance, is it possible to identify instances in which effects on the NE system are independent of those on the 5HT one. To do so, clorgyline, citalopram (when available), alprazolam, SAMe and nomifensine will be administered to patients with a focus on postsynaptic NE measures (urinary hydroxy-melatonin and beta lymphocyte receptors) and 5HIAA in the CSF.

2. Detailed investigation of factors controlling the elimination of NE and metabolites from plasma using deuterated intermediates synthesized at the NIH will be performed in volunteers so as to understand the apparent dissociation of results from those seen in urine in terms of antidepressant effects.

3. Studies will be performed in healthy volunteers age- and sex-matched to patients to assess acute noradrenergic and neuroendocrine responses to intravenous administraton of selective NE and serotonin uptake inhibitors.

4. SAMe will be administered to patients to see if it reverses the pretreatment abnormality of the NE system which we have recently documented (see Z01 MH-00447-16 LCS).

5. Pharmacokinetic studies will focus on possible active metabolites of alprazolam to see if these can explain their unexpected effects in man.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01855-01 LCS

PERIOD COVERED

October 1, 1984, through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Central Neurochemistry Service

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	0.2	3.5

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A joint laboratory effort has begun to provide development of new measures of neurotransmitters and their metabolites as well as more efficient delivery of previously established assays. The bulk of cerebrospinal fluid, plasma and urine assays within the intramural program of the NIMH are analyzed in this facility. Over 5000 samples were analyzed during the last year. Routine analyses include norepinephrine in all tissues, MHPG in CSF, plasma and urine, homovanillic acid and 5HIAA in CSF and plasma, and VMA in urine. Most of these assays can be performed by high performance liquid chromatography (HPLC) although urinary MHPG and VMA still require combined gas chromatography-mass spectrometry. New HPLC assays which have been developed for serotonin, dopamine, epinephrine, and normetanephrine in CSF, epinephrine and DOPAC in plasma and 5HIAA in urine. A GC-MS assay has been developed for normetanephrine. These new assays provide the means of studying interactions between neurotransmitter systems in man as well as distinguishing between differences in metabolism vs turnover.

PHS 6040 (Rev. 1/84) GPO 914-918

Other Professional Personnel:

Ivan N. Mefford	Special Expert	LCS/NIMH
Sanford P. Markey	Chief	AB/LCS/NIMH
David C. Jimerson	Chief	BP/LCS/NIMH
Markku Linnoila	Chief	LCS/NIAAA
Kenneth Kirk	Research Scientist	LC/NIADDKD

Project Description:

We have developed a structure for a centralized analytical laboratory focused on measures of neurotransmitters and their metabolites. This has evolved from two former components of the NIMH (Dr. Kopin's and Dr. Goodwin's) and a new component of NIAAA (Dr. Linnoila's). A professional (Dr. Ivan Mefford) has been recruited and arrived this July 1985 to direct the overall operation of the facility. Currently, four technicians from the NIMH and two from NIAAA develop and perform various assays, five on HPLC and one on GC-MS, in collaboration with the Section on Analytical Biochemistry (Dr. Markey). New assays are requested by clinical investigators in the NIMH and NIAAA through the Chief of the Section on Clinical Pharmacology, NIMH, and the Chief of the Laboratory of Clinical Studies, NIAAA.

Methods:

High performance liquid chromatography (HPLC) combined with electrochemical detection is the major method. The application of newly available microbore columns, more efficient multiple detector systems and automatic injectors allows for greater sensitivity and an expanded volume of samples. A particular emphasis is placed on the identification of appropriate internal standards synthesized by Dr. Kenneth Kirk in the Laboratory of Chemistry, NIADDKD.

Gas chromatography-mass spectrometry (GC-MS) is applied both to validate HPLC assays and to identify compounds for which HPLC analysis is not appropriate. More automated means of using GC-MS are being pursued.

Prior to application of either HPLC or GC-MS, complex, selective extraction and hydrolysis procedures are necessary. This is the most labor intensive aspect of performing several assays and requires techniques such as pre-columns, enzymatic acid hydrolysis, as well as differential organic extraction.

Findings:

1. Using coulometric vs ampermetric detection we are able to identify concentrations of epinephrine and serotonin in the 100 fentamoles/ml range in cerebrospinal fluid. These assays are just beginning to be applied to human material.

2. Concentrations of NE, MHPG, HVA, DOPAC and 5HIAA are measurable in the picomoles/ml range in CSF and/or plasma primarily by ampermetric detection. Thousands of samples have been analyzed and the results are described in reports from the various clinical investigators using these assays.

3. A GC-MS normetanephrine assay has been developed that provides the basis for the study of normetanephrine turnover following administration of its deuterated form. Initial studies are being performed in dogs.

Significance to Biomedical Research and to the Program of the Institute:

Neurotransmitter system function is implicated in major psychiatric illness, in behavioral medicine (e.g. responses to psychological and physiological stress) and in the mode of action of psychoactive as well as cardiovascular drugs. Improved methods for studying these neurotransmitter systems are crucial to understanding their operation in humans since adequate animal models or *in vitro* systems do not exist.

Only by fully and accurately quantitating neurotransmitters and their metabolites will it be possible to distinguish alterations of output vs those of metabolism and to relate amount to function. These techniques provide the best current hope of biochemically identifying individuals with psychiatric disease, at risk for such illness and/or most likely to respond to specific treatment.

Proposed Course:

With full-time professional direction of the laboratory, we plan to achieve the following over the next year:

1. Reduce the time necessary for routine assays.
2. Test the utility of plasma epinephrine vs norepinephrine in studies of stress.
3. Assess the usefulness of plasma MHPG vs plasma norepinephrine and normetanephrine as an index of noradrenergic function.
4. Measure free amines and metabolites in plasma and urine to study the renal clearance of these compounds.
5. Infuse deuterated normetanephrine to man to clarify whether it can be used as a selective measure of average norepinephrine release and to resolve controversies concerning the source of urinary metabolites of norepinephrine which provide the chief source of information in certain populations such as children.

Publications:
(in which personnel associated with the laboratory are cited but are primary products from other projects)

Hawley, R.J., Major, L.F., Schulman, E.A., Linnoila, M.: Cerebrospinal fluid MHPG and NE in alcohol withdrawal: Correlations with clinical signs. Arch. Gen. Psychiatry, in press.

Pickar, D., Labarca, R., Linnoila, M., Roy, A., Hommer, D., Everett, D., Paul, S.: Neuroleptic-induced decrease in plasma homovanillic acid: Correlation with antipsychotic activity in schizophrenic patients. Science, 225:954-956, 1984.

Garrick, N.A., Seppala, T., Linnoila, M., Murphy, D.L.: Rhesus monkey cerebrospinal fluid amine metabolite changes following treatment with the reversible monoamine oxidase type A inhibitor cimoxatone. Psychopharmacology, in press.

Zametkin, A., Rapoport, J.L., Murphy, D.L., Linnoila, M., Ismond, D.: Treatment of childhood attention deficit disorder with hyperactivity with monoamine oxidase inhibitors. Arch. Gen. Psychiatry, in press.

Korpi, E.R., Kleinman, J.E., Goodman, S.I., Phillips, I., DeLisi, L.E., Linnoila, M., Wyatt, R.J.: Serotonin and 5-hydroxyindoleacetic acid concentrations in different brain regions of suicide victims: Comparison in chronic schizophrenic patients with suicide as cause of death. Arch. Gen. Psychiatry, in press.

Post, R.M., Rubinow, D.R., Uhde, T.W., Ballenger, J.C., Lake, C.R., Linnoila, M., Jimerson, D.C., Reus, V.: Effects of carbamazepine on noradrenergic mechanisms in affectively ill patients. Psychopharmacology, in press.

Berrettini, W.H., Nurnberger, J.I., Scheinin, M., Seppala, T., Linnoila, M., Narrow, W., Simmons-Alling, S., Gershon, E.: Cerebrospinal fluid and plasma monoamines and their metabolites in euthymic bipolar patients. Biol. Psych., 20:257-269, 1985.

Garrick, N.A., Seppala, T., Linnoila, M., Murphy, D.L.: The effects of amiflamine on cerebrospinal fluid amine metabolites in the rhesus monkey. Eur. J. Pharmacol., 110:1-9, 1985.

DeLisi, L.E., Mirsky, A.F., Buchsbaum, M.S., van Kammen, D.P., Berman, K.F., Caton, C., Kafka, M.S., Ninan, P.T., Phelps, B.H., Karoum, F., Ko, G.N., Korpi, E.R., Linnoila, M., Scheinin, M., Wyatt, R.J.: The Genain quadruplets 25 years later: A diagnostic and biochemical followup. Psychiatry Res., 13:59-76, 1984.

Post, R.M., Rubinow, D.R., Uhde, T.W., Ballenger, C.J., Linnoila, M.: Dopaminergic effects of carbamazepine: Relationship to clinical response in affective illness. Arch. Gen. Psychiatry, in press.

Roy, A., Ninan, P., Mazonson, M.A., Pickar, D., van Kammen, D., Linnoila, M., Paul, S.M.: CSF monoamine metabolites in chronic schizophrenic patients who attempt suicide. Psychol. Medicine, in press.

Roy, A., Pickar, D., Linnoila, M., Doran, A., Paul, S.: Cerebrospinal fluid monoamine and monoamine metabolite concentrations and the dexamethasone suppression test: Relationship to life events. Arch. Gen. Psychiatry, in press.

Insel, T.R., Mueller, E.A., Alterman, I., Linnoila, M., Murphy, D.L.: Obsessive compulsive disorder and serotonin: Is there a connection? Biol. Psychiatry, in press.

Zametkin, A., Rapoport, J., Murphy, D., Linnoila, M., Karoum, F., Potter, W.Z., Ismond, D.: Urinary and plasma monoamines and metabolites in hyperactive children: Independence from behavioral state and persistence of effects post-amphetamine. Arch. Gen. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00787-06 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Mechanisms of Isolation Call in Squirrel Monkey (Saimiri sciureus)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. D. MacLean	Intramural Research Scientist	LCS, NIMH
Others:	J. D. Newman	Research Physiologist	LCE, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Comparative Ethology, NICHD

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The isolation call may rank as the most basic mammalian vocalization, serving originally to ensure maternal-offspring contact. Since the medial frontolimbic cortex has proved to be the main cortical area for eliciting vocalization in the squirrel monkey, the present project is concerned with testing the effects of damage to this area on the production of the isolation (separation) call. Adult squirrel monkeys of either sex are tested for their ability to produce isolation calls before and after ablations of different parts of the frontal lobe. Criterion performance is the production of 20 or more isolation calls during a 15-minute period of isolation in a sound-reducing chamber. This year's work has provided further evidence that lesions confined to a continuous band of paragenual and preseptal limbic cortex together with an as yet undefined adjacent area of medial frontal neocortex results in an enduring elimination of the spontaneous production of the call. Since lesions restricted to the different parts of the defined limbic area do not eliminate the call, the next step is to learn whether or not the medial frontal neocortex is necessary for the apparent concerted cortical action requisite for emitting the call. The relevance of this research to mental health is discussed.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: Work in our laboratory has been instrumental in revealing that the thalamocingulate division of the limbic system is implicated in three family related forms of behavior that characterize the evolutionary transition from reptiles to mammals--namely, (1) nursing, in conjunction with maternal care; (2) audiovocal communication for maintaining maternal-offspring contact; (3) and play. The thalamocingulate division represents the evolutionarily newest part of the limbic system which, in its totality, reflects an inheritance from early mammals. There is no counterpart of the thalamocingulate division in the brains of reptiles or other classes of vertebrates. Vocalizations used by mammals in maintaining maternal-offspring contact or contact with other individuals are known as isolation (separation) calls. In preceding work on this project, it was found that aspiration of the paragenual and preseptal cingulate cortex together with the adjoining medial frontal neocortex resulted in an enduring elimination of the spontaneous production of isolation calls. Since recent studies have revealed a co-innervation of the medial frontolimbic cortex by the anterior medial and parts of the medial dorsal and intralaminar nuclei of the thalamus, it has been the purpose of the present ongoing experiments to learn whether or not the production of the isolation call is dependent on the medial frontal neocortex.

Methods Employed: Adult male and female squirrel monkeys known as gothic and roman varieties (see M-NP-LI-17, 1964) are used for these studies. These two subspecies of squirrel monkeys with minor karyotypic differences can be behaviorally distinguished by their distinctive isolation calls as well as by their display behavior described in an accompanying report (Z01 MH 00851-21 LCS). The monkeys are tested for their ability to produce isolation calls before and after destruction of different parts of the brain. Criterion performance is the production of 20 or more separation calls during a 15-minute period of isolation in a sound-reducing chamber. Operated monkeys failing to achieve criterion are tested for an additional 15 minutes for their capability to emit responsive calls when listening to conspecific calls. Spectrographic analysis is used to compare the pre- and post-operative structure of calls. Cortical ablations are accomplished by subpial aspiration, while lesions of the brainstem are produced by electrocoagulation.

Major Findings: In the reports to be made on five monkeys, it has been the purpose in four to test the effects of lesions of discrete parts of the frontal lobe and, in the fifth animal, to coagulate the midline part of the medial dorsal nucleus where electrical stimulation has been reported to evoke peeping vocalization.

Frontal lobe lesions. One monkey (P-086) with a prefrontal lobectomy rostral to the cingulate sulcus continued to achieve criterion performance in the immediate post-operative period, as well as in the third post-operative month, but fell short of criterion in the fourth and final month of testing. Bilateral, symmetrical retrograde degeneration was observed in the dorsolateral sector of the medial dorsal nucleus caudal to AP 7.5 of the brain atlas.

In one monkey (R-5) described in a preceding report, aspiration of the paragenual and preseptal cingulate cortex, together with the greater part of

the medial frontal neocortex, eliminated the spontaneous production of the isolation call throughout eight months of testing. In monkey Z-5 (of this year's report), an attempt was made to replicate the ablation in R-5, except for restricting the neocortical lesion largely to area 32. The attempt was largely successful, except for an intrusion of the lesion on the left Nucleus accumbens. As in the case of R-5, there was an elimination of the spontaneous production of the isolation call. Also, as in R-5, there was no clearly detectable retrograde degeneration in the thalamus.

Because of earlier indications that the subcallosal and preseptal cingulate cortex may be of pivotal importance for the production of the call, an attempt was made to eliminate only this area of cortex. Because a direct surgical approach was impossible without damaging other areas, the ablation was performed by electrocoagulation. The lesion extended from the level of the genu to the septum, but there was a bilateral preservation of the cortex of the uppermost part of the subcallosal gyrus, including part of the taenia tecta, extending from about AP 17 to AP 15. There was no detectable degeneration in the thalamus. This animal continued to achieve criterion in the production of isolation calls throughout two months of testing.

Since the N. accumbens may be implicated in the separation call, an attempt has been made to destroy selectively this nucleus by electrocoagulation in monkey P-082. One week after surgery, this subject exceeded criterion in producing isolation calls; testing is being continued.

Midline thalamic lesion. Stimulation at the midline between the medial dorsal nuclei at AP 7 has been reported to evoke peeping vocalizations. In monkey A-6, an electrocoagulation was made at this level extending along the entire medial borders of MD. In serial brain sections the lesion measured about 1.5 mm in width and extended from about AP 7.5 to AP 5.5. This monkey continued to achieve criterion in the isolation call during four months of testing, but failed to emit display vocalizations (see accompanying report Z01 MH 00851-21 LCS).

Significance to Biomedical Research and the Program of the Institute: In the evolution of mammals, the ensurance of maternal-offspring contact was of the utmost importance: any prolonged separation from the mother is fatal for sucklings. It is presumed that the separation call evolved as a means of maintaining maternal-offspring contact. In psychiatry, the view is frequently expressed that traumatic separations in childhood may pave the way for many forms of mental illness. Because of the basic and stressful nature of the separation call, it is of fundamental interest for mental health to identify the brain structures involved in the call. Such interest is enhanced by the recognition that morphine eliminates the call, whereas the antagonist, Naloxone, reinstates the call.

The findings of this year's project provide further confirmation that a band of paragenual and preseptal limbic cortex and adjacent medial neocortex is essential for spontaneous production of the separation call. Experimentally, it appears that the production of the call depends upon the concerted action of the band of limbic cortex in question because ablations restricted to specific parts of it are not sufficient in themselves to eliminate the call. The limbic

cortex of this region belongs to the thalamocingulate division of the limbic system and is partially co-innervated by the anterior medial nucleus and parts of the medial dorsal nucleus. Apropos of the morphine-naloxone effects on the isolation call, it is of interest that, as shown elsewhere, the cingulate cortex of the rhesus monkey is rich in opiate receptors.

Findings in our laboratory have indicated that play is one of three family related behaviors represented in the cingulate gyrus. Since play is associated with laughter, the question arises as to the possibility of a reciprocal innervation of laughing and crying in the thalamocingulate division that might help to explain the enigmatic close relationship between laughing and crying and their alternation under certain conditions. With this in mind, a review of the clinical literature revealed a number of cases in which activation of different parts of the thalamocingulate division by irritative lesions or by electrical stimulation during therapeutic neurosurgery, resulted in moaning or crying or in smiling or laughter. Copious tearing was reported in some cases. The history of one patient implicated the medial frontolimbic cortex in both crying and laughter. There were isolated instances in which a playful expression or a subjective playful experience was identified with the cingulate gyrus.

Proposed course: To be continued.

Publications:

MacLean, P.D.: Brain evolution relating to family, play, and the separation call. Arch. Gen. Psychiatry 42: 405-417, 1985.

MacLean, P.D.: Evolutionary psychiatry and the triune brain. Psychol. Med. 15: 219-221, 1985.

MacLean, P.D.: Ictal symptoms relating to the nature of affects and their cerebral substrate. In Plutchik, R. (Ed.): Emotion: Theory, Research and Experience, Biological Foundations of Emotion. San Diego, Academic Press, Volume III (in press).

MacLean, P.D.: Culminating developments in the evolution of the limbic system: The thalamocingulate division. In Livingston, K. and Doane, B. (Eds.): Patterns of Limbic System Dysfunction. New York, Raven Press (in press).

MacLean, P.D.: The directional evolution of the limbic system (forthcoming).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00795-01 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparative Cytoarchitecture of the Cingulate Cortex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. D. MacLean Intramural Research Scientist LCS, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the present project is to provide additional cytoarchitectural information requisite for further comparative neurobehavioral studies concerned with clarifying the functional organization of the cingulate gyrus in regard to its apparent role in parental behavior, the separation call, and play. The present report based on 12 species of mammals, including the rock hyrax (Procavia capensis), supplies some new information regarding the retrosplenial cortex of the cingulate gyrus.

PHS 6040 (Rev. 1/84) GPO 904-917

Objectives: Cingulate, meaning girdle, applies to the part of the limbic lobe engirdling the corpus callosum. The term cingulate cortex, however, applies only to the cortex of the lobe peripheral to the taenia tecta and hippocampal formation. According to the growth ring hypothesis, the cingulate cortex represents an outgrowth from the hippocampus. Being a transitional cortex, it is otherwise referred to as mesocortex (M. Rose, 1926). Together with its thalamic connections, it will be referred to as the thalamocingulate division of the limbic system. There is no apparent counterpart of this subdivision in the reptilian brain. Work in this laboratory has been instrumental in showing that the thalamocingulate division is implicated in three forms of behavior that characterized the evolutionary transition from reptiles to mammals--namely, nursing, in conjunction with maternal care; audiovocal communication for maintaining maternal-offspring contact; and play. The limbic cortex comprises four distinctive cytoarchitectural areas. Based on histochemical findings such as those pertaining to acetylcholinesterase, opiate receptors, cholecystokinin, it is likely that different cingulate areas will prove to have a distinctive chemoarchitectonics. For selecting animals for future neurobehavioral research, it would be desirable to work with species in which particular cingulate areas are either unusually well-developed or poorly developed. With this in mind, the present project was undertaken so as to obtain additional data for judging this matter, focusing on the comparative cytoarchitecture of the retrosplenial area which has particularly distinctive features. Earlier work in our laboratory (e.g., Project No. M-NP-LI-31, 1967) has shown that units in the retrosplenial area of the squirrel monkey respond to photic stimulation, suggesting that this particular area is involved in visual functions.

Methods Employed: The present review is based on the examination of serial sections of 12 species of mammals in the brain collection of the Laboratory.

Major Findings: Brodmann (1906), in his original description, identified five retrosplenial areas that he labeled 29 a,b,c,d, and e. The most distinctive area is 29a, which, in eutherian mammals, is located in the fold of cortex just behind the splenium of the corpus callosum. The brain of the domestic rabbit (Oryctolagus cunniculus), belonging to the order Lagomorpha, provides an excellent illustration of the four main prototypical areas of the cingulate cortex. Examination in a rostrocaudal direction of the supracallosal parasplenial cortex reveals a thinning of the second layer and a progressive piling up of granular cells in layer III. This is area 29b. Behind the level of the splenium, rows of granular cells almost totally occupy the width of cortex identified as layers II and III. Immediately below this wide granular layer are layers V and VI. This area corresponds to 29a. Inferiorly, towards the subiculum, the superficial dark-staining cells of area 29a practically disappear as the cortex becomes transitional with rows of irregularly shaped cells that characterize the so-called retrosubicular cortex (area 48). Followed in a dorsal direction towards the parastriate cortex, the superficial granular layer becomes gradually thinner and deeper, forming a thin layer that corresponds to layer IV of the neocortex. In a lissencephalic brain such as that of the rabbit, a groove in the dorsolateral surface marks both the location of a blood vessel and the site of transition of the limbic cortex with the parastriate visual cortex. This groove probably corresponds to the splenial sulcus of macrosmatic gyrencephalic mammals.

In this project there was particular interest in examining the cingulate cortex of the hyrax (Procavia capensis, belonging to the order Proboscidea), because in this gyrencephalic mammal with special behavioral characteristics, most of the cingulate gyrus is surgically accessible directly from the dorsal surface. Moreover, for anatomical studies, there is the potential advantage that in this animal the limbic system is unusually well-myelinated. The hyrax brain has a moderately well-developed retrosplenial cortex that appears unique insofar as the granule cells of this area (and also of area 23) are the smallest yet encountered--being about 5 as compared with 7-8 for the mouse; 8-9 for the rat; 6 for the woodchuck (Marmota monax); 9-10 for the Syrian hamster; 7-10 for the Siberian hamster; 8-10 for the rabbit; 7-10 for the cat; 9-10 for the dog; 7-12 for the pygmy marmoset; and 7-10 for the squirrel monkey. It is to be noted that the common American opossum (Didelphis virginiana), a marsupial often referred to as a living fossil comparable to a Cretaceous mammal, does not show the same degree of granularization of the retrosplenial area.

In gyrencephalic sub-primate mammals, the retrosplenial cortex is bounded by the splenial sulcus and the retrosplenial cortex becomes transitional with the visual striate cortex in the retrosplenial stem of the sulcus. This stem compares to the rostral stem of the calcarine sulcus in primates. In primates, as in the Lagomorph brain, there is a line-up of the superficial granule cells in rows just behind the splenium. Rostrally, this superficial granular layer becomes transitional with the retrosubicular cortex, while caudolaterally, it becomes transitional with a deeper, thin granular layer that in turn meets the butt of the thick layer IV of the striate cortex. In horizontal sections, this transition is seen to occur within a distance of 1.5 mm. The picture is essentially the same in the pygmy marmoset.

Comment on the above findings will be deferred until the brains of more species have been examined.

Significance to Biomedical Research and the Program of the Institute: Further comparative neurobehavioral studies are required to clarify the functional organization of the cingulate gyrus in regard to its apparent role in parental behavior, the separation call, and play. The present project is intended to provide additional cytoarchitectural information requisite for such studies. The clinical and neuropharmacological relevance of work on the separation call has been commented upon in an accompanying report (Z01 MH 00787-06 LCS). In regard to the relevance of research on play, it is to be noted that play has been of unique importance in the evolution of mammals, and that in human beings it has contributed not only to acculturation with respect to games and sports, but also to creative associations generated by wit and humor in the various arts and sciences. Recent shuttle flights provide a timely illustration of the efficacy of play in relieving tension and tedium and in contributing to a sense of well-being.

Proposed Course: To be continued.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00847-05 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of the Neocortex in Coping with Complexity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: James L. Hill Guest Researcher LCS NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was inactivated earlier this year and was subsequently terminated in July, 1985 following the departure of the PI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00851-21 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Mechanisms of Display Behavior in Squirrel Monkey (Saimiri sciureus)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. D. MacLean	Intramural Research Scientist	LCS	NIMH
Others:	J. D. Newman	Research Physiologist	LCE	NICHD

COOPERATING UNITS *(if any)*

Laboratory of Comparative Ethology, NICHD

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In prosematic (nonverbal) communication, terrestrial vertebrates engage in four main forms of behavior known in ethological terms as displays. In the past, there existed almost no information about brain mechanisms involved in the performance of such displays. In a long-term, comparative neurobehavioral investigation dealing with this question, utilization has been made of the mirror display of gothic-type squirrel monkeys. This display incorporates features of the challenge, courtship, and signature displays of this species that have distinctive autonomic and somatic features. Earlier work on this project has shown that the internal pallidal segment of the striatal complex and its projecting pathways are essential for the performance of the display and that ascending pathways within the central tegmental tract are also involved. This year's experiments dealing with some unresolved questions concerning structures involved in the autonomic and somatic components of the display bring to a conclusion the phase of the investigation on brainstem mechanisms of the display.

Project Description:

Objectives: In prosematic (nonverbal) communication, terrestrial vertebrates engage in four main forms of behavior known in non-human animals as displays. In ethological language, such displays may be characterized as signature, challenge, courtship, and submissive displays. The present long-term project has utilized the mirror display typical of one variety of squirrel monkey for identifying brain structures essential for the performance of species-typical displays. The mirror display incorporates features of the signature, challenge, and courtship displays. In earlier phases of this project, it was shown that the medial globus pallidus is a site of convergence for neural systems required for performance of the mirror display. In anticipation of concluding the phase of this project concerned with the role of brainstem pathways, this year's experiments have included experiments intended to give further information in regard to structures respectively involved in the somatic and autonomic components of the display.

Methods Employed: Mature male gothic-type squirrel monkeys (see MNPLI 17, 1964) are used for these studies. Most males of this subspecies reliably display to their reflection in a mirror. A monkey is tested twice a day in its home cage in which the elevation of a panel reveals a full-length one-way mirror. The combined occurrence of the major components of the display--full genital tumescence, vocalization, and thigh-spreading--constitutes a trump display. After achieving criterion performance of a trump display in 80% of thirty trials, the monkey undergoes surgery for electrocoagulation of specific structures in the brainstem or subpial aspiration of cortical areas. Post-operative testing is continued until a subject achieves plateau performance in a series of at least three sets of thirty trials. Serial brain sections are prepared for histological study and reconstruction of the lesions.

Major Findings: Five monkeys have been used this year in combined studies on display behavior and on the isolation call (Z01 MH 00787-06 LCS). One of these animals (P-087) has not yet undergone surgery.

In one monkey (A-6) a narrow, vertical electrocoagulation involved a mid-line thalamic site at AP 6 where stimulation is known to elicit both penile erection and peeping vocalization (see also Z01 MH 00787-06 LCS). The lesion destroyed the entire innermost border of the medial dorsal nucleus from AP 7.5 to AP 5.5. Following surgery, there was a complete elimination of the display vocalization in five sets of 30 trials over a period of four months. During the first three months of testing, there was a gradual decline also in the thigh-spread component of the display, with performance at the zero level in the last two sets of trials.

The posterior part of the gyrus rectus contains limbic cortex where electrical stimulation is known to elicit full penile erection. Elimination of this sequestered cortex was successfully accomplished by electrocoagulation in monkey P-085. This subject continued to achieve full erection in each test throughout two months of testing. There was a statistically significant decline in the vocal and thigh-spread components of the display during the first set of 30 trials, with recovery in the next set of 30 trials. (See accompanying report cited above regarding lack of effect on the isolation call.)

One monkey (P-086) with a prefrontal lobectomy rostral to the cingulate sulcus continued to perform all components of the display during the first three months of testing (3 sets of trials), after which there was almost a complete absence of vocalization during the subsequent two sets of trials.

Finally, the paragenual and preseptal limbic cortex, together with the adjacent presulcal neocortex, was aspirated in a fourth monkey (Z-5). Except for a transient, statistically significant decline in vocalization, this subject continued to achieve criterion in the mirror display throughout five months of testing.

Summary. In concluding the phase of this work dealing with the brainstem, the following findings deserve emphasis: Based on systematic testing of more than 130 monkeys with lesions of various parts of the brain, it has become evident that interference with the performance of a mirror display occurs only with lesions involving either the medial segment of the globus pallidus and its projecting pathways, or following midbrain lesions of the central tegmental tract that (inferentially) interrupt ascending pathways to the striatal complex. Lesions of the medial preoptic area, the dorsomedial hypothalamic area, or the medial forebrain bundle interfere with the development of genital tumescence, but do not otherwise affect the performance of the display. Small hypothalamic lesions involving the origin of the medial longitudinal fasciculus selectively interfere with the display vocalization, and, as noted above, a midline thalamic lesion along the medial border of MD may result in a loss of such vocalization. No brainstem structures have been identified that specifically affect the thigh-spread component of the display. It is to be emphasized that bilateral destruction of large pathways such as the fornix, stria medullaris, mammillothalamic tract, and habenulopeduncular tract have no apparent effect on the performance of the display.

Significance to Biomedical Research and the Program of the Institute: Contrary to the traditional, popular claim that most human communication is verbal, students of behavior place a far greater emphasis on nonverbal communication that, as in the case of animals, involves four main forms of behavioral patterns. The cerebral structures accounting for the organization of such behavior have been largely unknown. Comparative neurobehavioral studies in this laboratory indicate that in animals as diverse as lizards and monkeys, parts of the striatal complex are essential for the performance of communicative displays, and may also be involved in conspecific recognition requisite for the initiation of displays.

Proposed course: The phase of this project dealing with brainstem structures involved in species-typical displays is to be completed with the publication of the results.

Publications:

MacLean, P.D.: Commentary: A brain theory commensurate with Procrustes' bed. The Behavioral and Brain Sciences 7: 344-345, 1984.

MacLean, P.D.: Fiber systems of the rat forebrain. In Paxinos, G. and Watson, C. (Eds.): The Rat Nervous System: Handbook for Neuroscientists. Australia, Academic Press (in press).

MacLean, P.D.: The triune brain. In Adelman, G. (Ed.): Encyclopedia of Neuroscience. Cambridge, Mass., Birkhauser Boston, Inc. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02219-02 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal Models of Anxiety

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. R. Insel	Staff Physician	LCS, NIMH
Others:	J. L. Hill	Expert Consultant	LCS, NIMH

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, NIMH; Clinical Neuroscience Branch, NIMH; Laboratory of Comparative Ethology, NICHD; Johns Hopkins University, Baltimore, MD; University of Colorado, Boulder, CO

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are investigating the neurobiology of anxiety from three approaches. We have used the anatomic method of quantitative in vitro autoradiography to map the regional distribution of the corticotropin releasing factor (CRF) receptor and the benzodiazepine receptor in brain. Using local microinjections into receptor dense areas, we have begun to test specific brain regions for behavioral effects relevant to anxiety. In a second approach, we have studied developmental antecedents of anxiety. Ultrasonic isolation calls in rodent pups are increased in anxious strains and appear selectively responsive to benzodiazepines. Finally, we have looked for differences in brain receptors in in "anxious" animals. Maudsley reactive rats have an increased number of adenosine receptors, but no difference in the number of benzodiazepine receptors compared to the Maudsley non-reactive strain. Studies in "anxious" rhesus monkeys reveal that the benzodiazepine receptor inverse agonist β-carboline-3-carboxylic acid-ethyl ester (β-CCE) has different behavioral effects depending on social context.

Other collaborative professional personnel engaged on the project:

M. Kuhar	Professor	Johns Hopkins U. Medical School, Baltimore, MD
S. Suomi	Chief	LCE NICHD
S. Maier	Professor	U. of CO, Boulder, CO
J. Glowa	Staff Scientist	NSB NIMH
P. Marangos	Section Chief	BPB NIMH

Project Description:

Objectives: This project, now in its second year, uses a comparative approach to investigate the neurobiology of anxiety. During the first year, behavioral studies in non-human primates explored the "anxiogenic" effects of inverse agonists for the benzodiazepine receptor and of the recently isolated peptide, corticotropin releasing factor (CRF). During this past year we have had three objectives:

(1) to localize brain regions where CRF and benzodiazepine receptor ligand effects are mediated;

(2) to construct a method to study the developmental antecedents of anxiety; and

(3) with these anatomic and developmental approaches to define neurobiological differences between animals selected for greater or lesser "anxiety."

Methods Employed: As much of our work in the past year required refinement of anatomic and developmental methods, we chose to temporarily shift most of our attention away from primates to use rodents during this phase.

For anatomic studies, we continued to use light microscopic receptor autoradiography (as described in Z01 MH 02219-01 CN) to localize brain receptors for CRF. Using the biologically active analogue ^{125}I-Nle 21-Tyr 32-CRF, we completed an extensive map of CRF receptors in rat brain and studied their regulation following adrenalectomy and selective hypothalamic lesions. In addition, we modified the autoradiographic method to permit simultaneous frozen sectioning of brains from different animals to obtain matched regions for quantitative comparisons of receptor binding. With this technique, we have been able to compare opiate, benzodiazepine, and adenosine receptor binding at the light microscopic level in the brains of animals selected for greater or lesser "anxiety."

To construct a method for the study of the developmental antecedents of anxiety, we needed two things: first, a consistent, quantifiable behavior present in infancy and second, a reliable measure of anxiety in adulthood. The infant behavior of greatest interest for us has been the ultrasonic isolation call of the rat pup. This call is representative of mammalian infant distress vocalizations, emerging immediately after birth and proving critical to eliciting maternal retrieval and attachment. We recorded ultrasonic calls

using a computer based digitizing system that allows both quantitation and characterization of each call. Pups were studied during two minutes of isolation. As a measure of anxiety or fearfulness in adulthood, we chose the open field test. Briefly, an individual rat is placed into a 1-meter diameter chamber and is observed for such spontaneous behaviors as exploration, locomotor activity, rearing, and defecation. Two animals can be studied simultaneously for social interaction. Both ultrasonic calls in infants and open field behavior in adults were used to test drugs and peptides believed to be relevant to anxiety.

As our work has focused more on the brain localization of peptide and drug effects, much attention has been given to local administration of "anxiogenic" compounds. Using the autoradiographic maps of CRF and benzodiazepine receptor distribution, we have begun to identify which regions mediate behavioral and endocrine effects by administering nanomolar quantities of drug or peptide into receptor "hot spots." This technique involves stereotactic surgical implantation of stainless steel guide cannulae into selected brain regions. For local injections awake animals are gently restrained while a 28-gauge injection cannula is inserted through the guide cannula and lowered to the appropriate depth. Injection volumes vary from .5 ul (for amygdala) to 3 ul (for lateral ventricle) and are delivered over 1 minute using a Harvard microinjection pump.

Finally, in order to define neurobiological differences between behaviorally different animals, we have followed two paths. In collaboration with Steven Suomi, we have extended our earlier β-CCE results to look at individual differences in drug responses of young rhesus monkeys selected for their responses to separation. Animals are studied both in isolation and in a social context; blindly rated for behavioral indices of fear, isolation, and aggression; and briefly restrained for plasma sampling. β-CCE (0.5 mg/kg or 0.05 mg/kg) or vehicle is administered intravenously following baseline behavior rating and plasma sampling. An extensive history of endocrine and behavioral responses to the stress of social separation has been previously recorded for each animal.

To focus more specifically on regional brain differences between animals genetically selected for fearfulness, we have studied Maudsley reactive and non-reactive rats. These are two congeneic inbred strains previously selected for differences in defecation and exploration, both indices of "emotional reactivity," in the open field apparatus. We have developed a colony of these two strains and used them in developmental and anatomic studies as summarized below.

Major Findings:

Localization of CRF and benzodiazepine effects. High affinity ($K_D \sim 3$ nM) brain receptors for CRF were discovered in discrete regions of the forebrain, particularly in lamina IV of the cortex, the median eminence, and the basolateral nucleus of the amygdala. Adrenalectomy down regulated pituitary CRF receptors approximately 50% but did not affect receptors in the forebrain. This finding suggests that these brain CRF receptors are regulated independently of the pituitary receptor. In an effort to define the role of these receptors, we

have replicated the behavioral results described by others with intraventricular administration of CRF (0.1 and 1 ug). We observed dose dependent decreases in exploratory behavior peaking at 1 hour following peptide administration. Preliminary results from injection of CRF (0.01 ug) into the basolateral aspects of the amygdala reveals a similar behavioral picture with a very rapid onset (< 5 minutes). In addition, preliminary results injecting the benzodiazepine, midazolam, into the same site suggest a full "anti-conflict" or anxiolytic effect. These studies then point to at least one site in the brain that may ultimately prove critical to the mediation of some of the effects of CRF and the benzodiazepines.

Developmental antecedents of anxiety. The ultrasonic isolation call has proven to be a robust and reproducible behavioral variable. We have shown that the call varies with age of the pup and ambient temperature. In addition, some mothers appear to consistently produce low-calling or high-calling litters. Drug studies have thus far revealed a selective effect of compounds that work via the benzodiazepine GABA receptor-Cl-channel complex. Diazepam (0.15 - 1.0 mg/kg) decreases the isolation call without decreasing locomotor activity. Pentylenetetrazol (20 mg/kg) and the benzodiazepine receptor inverse agonist FG7142 (25 mg/kg) increase the number of calls during 2 minutes of isolation. Although other agents, such as morphine, can affect the number of calls, these effects appear to be secondary to sedation as locomotor activity decreases with the decrease in vocalization. Curiously both acute and chronic treatments with imipramine, 6-hydroxydopamine, or CRF do not affect the number or quality of the isolation calls. There is a profound difference in the number of isolation calls given by Maudsley reactive and Maudsley non-reactive pups. Even by the second day of life, reactive pups emit fivefold more isolation calls during 2 minutes of separation. This difference is not affected by cross-fostering the pups on the day of birth and it remains consistent through the first week of life.

Neurobiological differences relevant to anxiety. Maudsley reactive and non-reactive strains can be consistently distinguished by exploration and defecation in the open field apparatus. This difference like the difference in infant vocalization rate, is not affected by cross-fostering and is stable across three generations. Contrary to a previous report, these strains do not differ in the number or affinity of brain benzodiazepine receptors assessed by both homogenate and quantitative autoradiographic techniques, nor do they differ in their sensitivity to the convulsant pentylenetetrazol. Studies of strain differences in GABA modulaton of the brain benzodiazepine receptor are underway. Curiously, Maudsley reactive rats show a 20-40% localized increase in adenosine receptors and the adenosine agonist, 1-phenylisopropyladenosine, partially reverses behavioral differences between the two strains.

Administration of β-CCE to socially housed rhesus monkeys is showing us that our previous concepts of the behavioral effects of the drug were overly simplistic. There are great individual differences in the response to this drug, ranging from increases in aggression to isolation. These behavioral differences depend, in part, on the social rank of the individual, as well as on the social context in which the drug is given (e.g. isolation vs. peer group).

Significance to Biomedical Research and the Program of the Institute: These studies provide a comprehensive strategy to study the neurobiology of anxiety and fear. The developmental approach helps to define constitutional elements of fearfulness that are clearly relevant to clinical studies of Childhood Separation Disorder, Panic Disorder, and other anxiety syndromes. The evidence of a role for the benzodiazepine receptor in separation (and attachment) behavior underlines the importance of constitutional factors in the integration of early experiences. By defining brain regions, as well as receptor systems important to the mediation of anxiety, these studies begin to demonstrate a neurochemical anatomy for the mediation of responses relevant to both pathophysiologic and normal anxiety.

Proposed Course: Localization studies will continue to follow autoradiographic maps to look for regions that mediate the behavioral effects of CRF and benzodiazepine receptor ligands. A major focus in the coming year will be the local administration of antisera to CRF, to assess the physiologic and behavioral effects of local reductions in CRF concentrations. Similarly, the use of benzodiazepine receptor antagonists will provide a tool for studying the behavioral consequences of selective, local decreases in the presence of endogenous or exogenous benzodiazepine receptor ligands.

For both the localization and the developmental studies we hope to graduate from rodents to non-human primates. Our colony of pygmy marmosets, reproducing since May 1984, is finally stable enough to permit separation and drug administration studies. These active, pair bonding primates should provide an ideal species for both behavioral and anatomic studies.

Studies of the neurobiological differences in Maudsley reactive and non-reactive rats will continue with assays of monoamines and their metabolites in selective brain regions and comparisons of CRF and adrenergic receptor binding.

Primate studies with β-CCE will continue in Madison, Wisconsin, until the new NICHD-NIMH primate facility opens in Poolesville. We look forward to this Center opening by the summer of 1986.

Publications:

DeSouza, E.B., Insel, T.R., Perrin, M.H., Rivier, J., Vale, W.W., and Kuhar, M.J.: Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: An autoradiographic study. J. Neuroscience (in press).

DeSouza, E.B., Insel, T.R., Perrin, M.H., Rivier, J., Vale, W.W., and Kuhar, M.J.: Differential regulation of corticotropin-releasing factor (CRF) receptors in centerior and intermediate lobes of pituitary and in brain following adrenalectomy in rats. Neurosci. Ltrs. (in press).

DeSouza, E., Perrin, M., Insel, T.R., Rivier, J., Vale, W., and Kuhar, M.: Corticotropin-releasing factor receptors in rat forebrain: Autoradiographic identification. Science 224: 1449-1451, 1984.

Insel, T.R.: The neurobiology of anxiety: A tale of two systems. Proceedings of the Clark Institute Symposium on Anxiety Disorders. (in press)

Insel, T.R., Ninan, P., Aloi, J., Jimerson, D., Skolnick, P., and Paul, S.: A novel benzodiazepine receptor mediated model of anxiety. Studies in non-human primates. Arch. Gen. Psychiatry 41: 741-750, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00382-11 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Localization and Characterization of Brain Neuropeptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Gerhard Skofitsch	International Research Fellow (Fogarty International Center)	LCS, NIMH
Nadav Zamir	Visiting Associate	LCS, NIMH
Robert Eskay	Neurochemistry Section	LCS, NIAAA
Yosef Tizabi	Assoc. Prof., Department of Pharmacology Howard, University	

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LAB/BRANCH Laboratory of Clinical Science

SECTION Histopharmacology

INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.3	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) Two types of corticotropin releasing factor (CRF)-like immunoreactivity have been identified in the rat brain and biochemically characterized. One type is obtained in the hypothalamus in fundibular system, the other one in the sensory hindbrain area and the spinal cord. CRF receptors have been identified in the spinal trigeminal and superficial spinal cord areas strengthening the latter suggestion of a presence of CRF in extrahypothalamic primary sensory areas. 2) Capsaicin treatment of newborn rats was previously shown to cause selective degeneration of primary sensory neurons and a depletion of substance P, somatostatin, cholecystokinin and vasoactive intestinal polypeptide from central and peripheral sensory nerves. We now add CRF, calcitonin gene related peptide (CGRP) and galanin (GA) to the list of capsaicin sensitive sensory neuropeptides. CGRP has been shown to be a potent vasodilator and thus seems to be of major interest in blood pressure control. 3) Immmunohistochemical distribution of CRF, atrial natriuretic factor (ANF), CGRP, GA and melanin concentrating hormone (MCH) has been demonstrated in the rat brain and stereotaxic atlases were prepared. 4) Using radioimmunoassay (RIA) and the micropunch technique quantitative distribution of CRF, ANF, CGRP and MCH-like peptides in about 50 brain nuclei has been determined. 5) Using RIA and high performance liquid chromatography (HPLC) CRF, MCH, CGRP antisera were biochemically characterized. 6) The autoradiographic distribution of CRF and CGRP receptor sites in the rat brain have been demonstrated.

Project Description

Objectives: 1) Localization and identification of rat and ovine corticotropin releasing factor (CRF)-, atrial natriuretic factor (ANF)-, calcitonin gene related peptide (CGRP)-, galanin (GA)-, and melanin concentrating hormone (MCH)-like immunoreactivity in the rat central and peripheral sensory nervous system. 2) Biochemical characterization of rat and ovine CRF-, ANF-, CGRP-, and MCH-antisera. 3) Characterization of CRF and CGRP receptors in the rat brain. 4) Pharmacologically induced alterations of central and peripheral peptidergic systems.

Methods Employed: 1) Indirect immunohistochemistry of CRF, ANF, CGRP, GA and MCH. 2) Radioimmunoassay of CRF, ANF, CGRP, GA and MCH. 3) High performance liquid chromatography of synthetic peptides and homogenates of microdissected brain nuclei. 4) Microdissection of brain nuclei. 5) Stereotaxically placed microinjections of retrogradely transported fluorescent dyes. 6) Selective pharmacologically induced degeneration of primary sensory neurons or depletion of catecholaminergic nerves of their transmitter substances. 7) Autoradiography of rat brain sections.

Major Findings:

(A) Immunohistochemistry revealed that there are discrete regions of the rat brain where CRF immunoreactive cell bodies and fibers are seen with ovine and rat CRF antisera (e.g., the bed nucleus of the stria terminalis, the paraventricular and dorsolateral tegmental nuclei) whereas there are cell bodies and fibers (e.g. in the cortex) which could be seen with antisera directed to rat CRF only, or terminal fields (e.g. in the spinal trigeminal tract and nucleus, the substantia gelatinosa or the spinal cord) which could be visualized with ovine CRF-antisera only.

Tissue concentrations obtained by RIA of micropunched brain areas with the four antisera were frequently dissimilar. High performance liquid chromatography and RIA of rat brain tissue samples revealed together with immunocytochemical preadsorption techniques, that there is more than one form of CRF-like immunoreactivity in the rat brain. There is indirect evidence that besides the rat CRF-like material in the hypothalamic area there is a peptide in the medulla and spinal cord which cross-reacts with antisera directed to ovine CRF only. The "ovine CRF-like material" was shown to be colcalized with subtance P, vasoactive intestinal polypeptide, cholecystokinin and Leu-enkephalin in primary sensory neurons and ganglia and to be sensitive towards treatment of rats with capsaicin, which is known to cause degeneration of primary sensory neurons.

Using autoradiographic methods with rat or ovine ^{125}I-Tyr°-CRF the ovine ligand exclusively was able to show receptor sites as was published for the rat forebrain. However we also found specific binding in the caudal spinal trigeminal area, the nucleus tractus solitarii, the substantia gelatinosa and the dorsal horn of the spinal cord (laminae I and II), indicating that a CRF-like peptide might be involved in the modulation of peripheral nociceptive information as dense accumulations of binding sites occur at sites known to be rich in nerve endings of primary sensory neruons.

Chronic treatment of rats with reserpine caused a 40% reduction in the CRF-content of the median eminence and the posterior pituitary while treatment with desmethylimipramine caused a 61% rise in the CRF content of the posterior pituitary. The results support the role of monoaminergic regulation of CRF release from the median eminence and further suggests an interaction between monoaminergic and CRF containing neurons in the posterior pituitary.

(B) Atrial natriuretic factor (ANF): Using an antiserum directed against rat atriopeptin III the immunohistochemical distribution of atrial natriuretic factor (ANF)-like immunoreactivity in the rat central nervous system was demonstrated. Cell bodies were observed in the telencephalon (nucleus interstitialis striae terminalis and in the amygdala), throughout the diencephalon in all nuclei of the "anteroventral third ventricle " (AV3V), the base of the hypothalamus, the area ventral to the zona incerta, the medial forebrain bundle and the medial habenula, in the mesenaphalon (mammillary body, substantia nigra lateralis, dorsal and ventral parabrachial nuclei) and very sparse in the medulla oblongata along the fourth ventricle towards the vestibular nuclei, the nucleus tractus solitarii and nervi trigemini. Fibers were present wherever cell bodies were located. The highest relative densities were observed in the AV3V area and the medial habenula. Sparse fibers were also seen in the spinal cord (dorsal and ventral horn and around the central canal) and in the posterior pituitary. The immunohistochemical results could be verified by radioimmunoassay of 47 microdissected brain areas.

(C) Galanin (GA): Using an antiserum in rabbits against synthetic galanin (GA) and the indirect immunofluorescence method, the distribution of GA-like immunoreactive cell bodies and nerve fibers was studied in the rat central nervous system (CNS) and a detailed stereotaxic atlas of GA-like neurons was prepared. GA-like immunoreactivity was widely distributed in the rat CNS. Appreciable numbers of GA-positive cell bodies were observed in the rostral cingulate and medial prefrontal cortex, the nucleus interstitialis striae terminalis, the medial preoptic, preoptic periventricular, and preoptic suprachiasmatic nuclei, the medial forebrain bundle, the supraoptic, the hypothalamic periventricular, the paraventricular, the arcuate, dorsomedial, perifornical, thalamic periventricular, anterior dorsal and lateral thalamic nuclei, the medial and central amygdaloid nuclei, dorsal and ventral premaillary nuclei, at the base of the hypothalamus, in the central gray matter, the hippocampus, the dorsal and caudoventral raphe nuclei, the interpeduncular nucleus, the locus coeruleus, ventral parabrachial, solitarii and commissuralis nuclei, in the A1, C1 and A4 catecholamine areas, the posterior area postrema and the trigeminal and dorsal root ganglia. Fibers were generally seen where cell bodies were observed. Very dense fiber bundles were noted in the septohypothalamic tract, the preoptic area, in the hypothalamus, the habenula and the thalamic periventricular nucleus, in the ventral hippocampus, parts of the reticular formation, in the locus coeruleus, the dorsal parabrachial area, the nucleus and tract of the spinal trigeminal area and the substantia gelatinosa, the superficial layers of the spinal cord and the posterior lobe of the pituitary. The localization of the GA-like immunoreactive neurons in the rat CNS suggests a partial coexistence with catecholaminergic neurons as well

as a possible involvement of the GA-like peptide in a neuroregulatory role. Furthermore, we have localized GA-like immunoreactivity in capsaicin sensitive neurons in the trigeminal and dorsal root ganglia, in the superficial layers of the dorsal spinal cord (laminae I and II), the substantia gelatinosa, the nucleus and tractus of the spinal trigeminal nerve and the nucleus commissuralis. Since it is well documented that capsaicin causes selective degeneration of primary sensory neurons of the C-fiber type and type B-cells of sensory ganglia, it is suggested that GA in capsaicin sensitive nerves represents a novel peptidergic system possibly involved in the transformation or modulation of peripheral nociceptive impulses.

(D) Calcitonin gene-related peptide (CGRP): Using an antiserum against synthetic human calcitonin gene-related peptide (CGRP) the distribution of CGRP-like immunoreactive cell bodies and nerve fibers was studied in the rat central nervous system. A detailed stereotaxic atlas of CGRP-like neurons was prepared. CGRP-like immunoreactivity is widely distributed in the rat central nervous system. CGRP positive cell bodies were observed in the preoptic area and hypothalamus (medial preoptic, periventricular, anterior hypothalamic nuclei, perfornical area, medial forebrain bundle), the premamillary nucleus, amygdala medialis, hippocampus and dentate gyrus, central gray and a region in the thalamus (medial to the lemniscus medialis). In the midbrain a large cluster of cells was contained in the peripeduncular area ventral to the medial geniculate body. In the hindbrain cholinergic motor nuclei (III, V, VI, VII, XII) contained CGRP-immunoreactivity. Cell bodies were also observed in the ventral tegmental nucleus, the parabrachial nuclei, superior olive and nucleus ambiguus. The ventral horn cells of the spinal cord, the trigeminal and dorsal root ganglia also contained CGRP-immunoreactivity. Dense accumulations of fibers were observed in the amygdala centralis, caudal portion of the caudate putamen, sensory trigeminal area, substantia gelatinosa, dorsal horn of the spinal cord (laminae I and II). Other areas containing CGRP-immunoreactive fibers are the septal area, nucleus of the stria terminalis, preoptic and hypothalamic nuclei (e.g., medial preoptic, periventricular, dorsomedial, median eminence) medial forebrain bundle, central gray, medial geniculate body, peripeduncular area, interpeduncular nucleus, cochlear nucleus, parabrachial nuclei, superior olive, nucleus tractus solitarrii, and in the confines of clusters of cell bodies. Some fibers were also noted in the posterior pituitary and the sensory ganglia. The immunohistochemical data could be confirmed by radioimmunoassay of 47 brain nuclei. The antiserum to CGRP was thoroughly characterized using high performance liquid chromatography, RIA and parallel displacement.

Further immunohistochemical studies revealed that CGRP-like immunoreactivity in the spinal trigeminal nucleus and tract, the substantia gelatinosa and the dorsal spinal cord as well as the trigeminal and dorsal root ganglia is markedly depleted by capsaicin known to cause selective degeneration of a certain population of primary sensory neurons. Furthermore, CGRP and substance P-like immunoreactivity was shown to be colocalized in capsaicin sensitive fibers and to coexist in a certain number of cell bodies of the trigeminal ganglion and the spinal dorsal root ganglia.

As substance P has been suggested to be a transmitter substance of peripheral nociceptive fibers, the physiological significance of the coexistence of CGRP and substance P in primary sensory neurons has to be further evaluated.

The vasodepressor and plasma extravasating activity of CGRP was compared to that of substance P in pithed, vagotomized rats. Systemic administration of CGRP caused a long lasting vasodilatation accompanied by a parallel rise in heart rate which persists after β-adrenoceptor blockade and this indicates a direct action on the heart. For any equimolar dose of CGRP the hypotensive effect was much larger than to substance P. In contrast, after systemic administration of equimolar doses CGRP was much less effective in producing vasodilatation than substance P. Therefore, at equimolar doses CGRP is 10 times more potent than subtance P in producing vasodilatation but it posesses less than a third of the potency of substance P in producing plasma extravasation.

Using cryostat sections of the rat brain mostly containing cerebellum and ^{125}I-Tyr°-rat CGRP in autoradiographic methods, scatchard analysis revealed a single class of receptors (R=0.94) with an equilibrium dissociation constant (K_D) of 0.96 ± 0.36 nM and a maximal number of binding sites of 76.4 ± 13.3 fmol/mg protein. Based on these results the autoradiographic distribution of CGRP-like binding sites in the rat brain was demonstrated. High densities of CGRP receptor sites were observed in the medial prefrontal and posterior medial and basal temporal cortices. A dense continuity of receptors was seen beginning at the nucleus accumbens, the ventral caudate putamen, in the central and lateral amygdaloid nuclei. Other dense receptor areas are the organuum vasculosum laminae terminalis (OVLT), the subfornical organ, the superior colliculus, the central parts of the inferior colliculus, the medial geniculate body, the dorsal raphe, the inferior olive, the nucleus tractus solitarii, the nucleus commissuralis, the nucleus of the twelfth nerve, the fasciculus cuneatus, the dorsal horn of the spinal cord and the area around the central canal. Low to moderate receptors were observed in the insular and piriform cortices, the gray matter of the hippocampus, the septal and preoptic areas, the anterior ventral and periventricular thalamic nuclei, the medial hypothalamic area, the central gray, the reticular part of the substantia nigra, the lateral lemniscus, the dorsal tegmental area, the spinal trigeminal and parvocellular reticular nuclei, the nucleus of the fifth nerve and the gray matter of the ventral spinal cord.

As with other newley described brain neuropeptides it can only be conjectured that CGRP has a neuroregulatory action on a variety of functions throughout the brain and spinal cord.

(E) Melanin Concentrating Hormone (MCH): Using antisera generated in rabbits against salmon melanin concentrating hormone (MCH) coupled to human thyroglobulin, the distribution of MCH-like immunoreactivity was mapped throughout the rat central nervous system. The distribution of MCH-like immunoreactivity in rat brain is unique and different from the distribution of other neuropeptides. Cell bodies are located mainly in the medial fore-

brain bundle and in proximity to well defined hypothalamic nuclei. Fibers were seen throughout the rat brain in all neocortical areas, the eostriatum and the amygdala, in the diencephalon in most hypothalamic nuclei, the habenula, the mamillary body and very dense in the medial forebrain bundle and just ventral to the zona incerta ("subzona incerta"). In the mesencephalon there are fibers in the central gray; in the pons fibers are contained in the dorsal and ventral parabrachial nuclei; in the tegmental area ventral to fourth ventricle; in the medulla oblongata, in the spinal trigeminal area, the substantia gelatinosa and the reticular nuclei. In the spinal cord there are more fibers in the dorsal than in the ventral horn. The posterior pituitary also contained few MCH-like fibers. MCH-like immunoreactive perikarya and fibers are predominant in the posterior hypothalamic area, mostly in the medial forebrain bundle - lateral hypothalamic area, subzona incerta and the perifornical area. The immunohistochemical distribution could be verified by radioimmunoassay of 42 microdissected brain areas. Analysis of rat brain extracts by high performance liquid chromatography and radioimmunoassay revealed that synthetic salmon MCH elutes more than 15 min prior to the MCH-like immunoreactive material in the rat brain. However, using parallel displacement techniques in RIA synthetic salmon MCH and tissue extracts displaced the ^{125}I-salmon MCH in a parallel shaped dose response curve from the antiserum. It is suggested that a peptide similar, but not identical, to salmon MCH is present in the rat brain.

The physiological role of the MCH-like material in the rat brain is unknown. However, its existence in the mamallian hypothalamo-neurohypophyseal system suggests a role in posterior pituitary function, distinct from the mediation of color change seen in lower vertebrates. Indeed, an osmotic stimulus (2% NaCl as drinking fluid for 120 hrs) which is associated with enhanced secretory activity from the neurohypophysis caused significant increases in MCH-like concentrations in the lateral hypothalamic area (LHA; which includes the MFB) and in the neurointermediate lobe, (LHA: 379.0 ± 55.0 fmole/mg protein in controls vs 770.0 ± 91.0 fmol/mg protein in salt loaded rats; Neurointermediate Lobe: 294.0 ± 39.0 fmole/mg protein in controls vs 732.0 ± 63.0 fmole/mg protein in salt loaded animals; mean ± SEM, n=8, $p < 0.01$ in each case).

Since MCH containing neurons originate exclusively from the hypothalamus to innervate the pituitary gland, hypothalamus and many extrahypothalamic brain regions, MCH may be well situated to coordinate complex functions such as regulation of food and water intake.

Significance to Biomedical Research and the Program of the Institute: The basic neuroanatomical and methodolgical studies reported here lay the groundwork for further studies on the role of CRF, ANF, CGRP, GA and MCH in the central and peripheral nervous system.

The predominance of ANF-like perikarya and fibers in the AV3V area suggests an involvement of this peptide in central blood pressure regulation. Preliminary study revealed ANF microinjected in the rat brain produce substantial increases in both BP and HR.

The presence of CGRP in motor neurons and especially the ventral horn cells of the spinal cord is of great clinical significance since this is the first peptide to be found to exist in the cholinergic motor nerves that innervate skeletal muscle. The possible modulatory influence of CGRP on the release of acetylcholine at the motor end plates is very important to investigate and may be important in a variety of muscle syndromes.

Proposed Course of the Project: 1) The involvement of central CRF in stress will be evaluated. 2) Attempts are in progress to identify the physiological significance of capsaicin sensitive central and peripheral neuropeptides (e.g. substance P, somatostatin, cholecystokinin, vasoactive intestinal polypeptide, CRF, CGRP, GA) on peripheral cardiovascular mechanisms. 3) The central role of ANF as a regulator of blood pressure and the mode of action is being evaluated. 4) CGRP is believed to be also contained in cholinergic motor-neurons. The influences of this peptide on peripheral cholinergic motor function has to be determined. 5) CGRP has been shown to be a potent vasodilator, the most powerful vasodilating neuropeptide known at present. Its role in blood pressure control will be studied. 6) Coexistence of GA with catecholoamines in certain brain areas (locus coeruleus) is interesting. The influences of GA on catecholaminergic function has to be determined. A sensitive radioimmunoassay to GA will be developed in order to study the influence of locus coeruleus lesions on GA concentration in the cortex.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00388-09 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Coexistence of Peptides and Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Hideaki Hara	Guest Researcher	LCS, NIMH
Jacqueline N. Crawley	Chief, Unit on Behavioral Neuropharmacology	NSB, NIMH

COOPERATING UNITS (if any)

Clinical Neuroscience Branch

LAB/BRANCH

Laboratory of Clinical Science

SECTION

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INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) The distribution and density of vasoactive intestinal polypeptide (VIP) immunoreactive acetylcholinesterase (AChE)-containing nerves around the cerebral arteries was studied by using whole mounts with or without lesioning the sphenopalatine ganglia. Abundant VIP and AChE-containing nerves were observed around the cerebral blood vessels in normal rats. VIP and AChE-staining was also demonstrated in neurons within the sphenopalatine ganglia. Lesions of the sphenopalatine ganglia resulted in a marked reduction of both VIP and AChE activity. In many neurons, coexistence of both VIP and AChE was revealed. These results demonstrate that cholinergic neurons from the sphenoplatine ganglia innervate the cerebral vasculature at the base of the brain, and that VIP and AChE coexists within the same nerve fibers. These results reveal that the major cholinergic innervation to the cerebral blood vessels emanates from the sphenopalatine ganglion and that VIP coexists within these neurons. (2) Colocalization of substance P (SP), corticotropin releasing factor (CRF), and AChE was detected by retrograde tracing and immunocytochemical staining in the nucleus tegmentalis dorsalis lateralis (ntdl) projecting to the medial frontal cortex (MFC), septum, and thalamus of the rat. These results suggest that SP and CRF coexist within a subpopulation of ntdl cholinergic neurons that project to a number of forebrain regions including the MFC. Behavioral studies of the effects of SP, CRF, and the cholinergic agonist, carbachol, employed microinjections into the MFC of rats. SP and CRF did not elicit any behavioral effects when administered alone. Carbachol produced a stereotyped motor behavior, consisting of rapid forepaw treading while in an upright posture, resembling "boxing". SP increased carbachol-induced "boxing". CRF decreased carbachol-induced "boxing". One possible functional significance of the coexistence of SP, CRF, and acetylcholine, in neurons projecting to the medial frontal cortex in rats, appears to be a modulatory potentiation of cholinergic response by SP, and a modulatory inhibition of the cholinergic reponse by CRF.

Project Description:

Objectives: (1) To explore the origin of the cholinergic/VIP innervation to the major cerebral arteries of the rat brain. (2) To investigate the possibility that SP, CRF and AChE are colocalized within the ntdl neurons projecting to the MFC. To undertake studies to test the possibility of a potential behavioral interaction of SP, CRF and acetylcholine.

Methods Employed: (1) Stereotaxic lesions of the sphenopalatine ganglia. (2) Immunohistochemistry of VIP, SP, CRF. (3) AChE histochemistry. (4) Retrograde fluorescent tracer procedure. (5) Microinjection of peptides and carbachol into indwelling cannulae implanted in the MFC. (6) Recording of behavioral observations.

Major Findings:

(A) A similar density and distribution of the VIP immunoreactive and AChE-containing nerves in various proteins of the rat cerebral vasculature was observed. VIP immunoreactivity and AChE activity coexists in the same sphenopalatine ganglion cells and nerves innervating the cerebral blood vessels. A marked decrease in arterial VIP immunoreactivity and AChE staining was observed on the ipsilateral side following unilateral lesions of the sphenopalatine ganglion.

(B) Immunocytochemical and retrograde tracing experiments suggest the coexistence of subtance P, corticotropin releasing factor, and acetylcholinesterase in cell bodies of the nucleus tegmentalis dorsalis lateralis which project to the medial frontal cortex of the rat. Carbachol elicited a dose-dependent "boxing"-like behavior when injected into the rat MFC. Behavioral experiments revealed that substance P increased the response to the cholinergic agonist, carbachol, in the stereotyped motor pattern resembling "boxing", while CRF decreased the response to carbachol, when injected into the medial frontal cortex. Neither peptide had behavioral effects when administered alone. Substance P and CRF appear to have functional significance as modulators of acetylcholine in one terminal region of triple coexistence.

Significance to Biomedical Research and the Program of the Institute: (1) The knowledge that VIP is contained in cholinergic neurons that control cerebral circulation will aid in revealing the mechanisms by which the brain controls its blood supply, and ultimately the treatment of cerebrovascular diseases. (2) The coexistence of SP, CRF and ACh in nerves that innervate the medial frontal cortex raises questions about the process of neurotransmission and may have important implications for our understanding and treatment of mental disorders.

Proposed Course of the Project: Further studies of the influence of cortical peptides on the "boxing" phenomenon resulting from carbachol injection into the MFC are in progress.

Publications:

1. Hara, H., Hamill, G. S. and Jacobowitz, D. M. Origin of cholinergic nerves to the rat major cerebral arteries: Coexistence with vasoactive intestinal polypeptide. Brain Res. Bull., 14: 179-188, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00396-07 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Study of Proteins Within the CNS by Two-Dimensional Gel Electrophoresis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

William E. Heydorn	Staff Fellow	LCS, NIMH
David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Matthew A. Sills	Guest Researcher (NIGMS Fellow)	NIGMS & LCS NIMH
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COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using two-dimensional gel electrophoresis we have continued our studies of proteins in the central nervous system. First, we have shown that a number of proteins are altered in concentration in the parietal cortex, hippocampus and cerebellum following either bilateral lesioning of the locus coeruleus or neonatal treatment with 6-hydroxydopamine. Second, we have demonstrated that chronic administration of the type A monoamine oxidase inhibitor clorgyline to rats for three weeks produces a change in the relative amount of five proteins in the parietal cortex but only a single protein in the hippocampus. Third, we have determined the subcellular localization of about 50% of the proteins visible on two-dimension gels generated using rat brain. Fourth, we have identified eight distinct proteins within the subfornical organ of the rat that are altered in relative turnover rate by salt loading and/or water deprivation. Fifth, using samples of astrocytoma obtained during surgery, we have shown that there is a general consistency in the protein pattern among the twenty samples studied. However, there are marked quantitative as well as qualitative differences in the two-dimensional protein patterns of astrocytomas compared to normal human cortex. Sixth, we have identified an apparent genetic polymorphism for a protein which is present in the hypothalamus of Sprague-Dawley rats. Finally, we have identified the location of both the α and β subforms of soluble glutamic oxaloacetic transaminase on two-dimension gels of rat and human cortex.

Project Description:

Objectives: 1) Determine the role that the catecholamine neurotransmitter norepinephrine plays in regulating the concentration of individual proteins in discrete areas of the CNS. 2) Investigate the effect of chronic treatment of rats with the type A monoamine oxidase inhibitor clorgyline on the concentration of individual proteins in the parietal cortex and the hippocampus. 3) Determine the subcellular localization of proteins present on two-dimensional gels of rat cortex. 4) Identify proteins within the subfornical organ of the rat whose rate of synthesis and/or degredation is affected by either salt loading or water deprivation. 5) Compare quantitatively and qualitatively the two-dimensional protein pattern of astrocytomas to that seen in normal human cortex. 6) Investigate the occurence of a genetic polymorphism in the hypothalamus of a group of inbred Sprague-Dawley rats. 7) Identify various proteins which are visible on two-dimension gels of rat and human cortex.

Methods Employed: 1) Two-dimensional polyacrylamide gel electrophoresis; 2) Photochemical silver staining of proteins on polyacrylamide gels; 3) Computerized scanning densitometry of proteins on two-dimensional gels; 4) Microdissection of discrete regions of the rat brain; 5) Electrophoretic transfer of proteins to nitrocellulose paper and subsequent identification of proteins by use of specific antisera; 6) Radiolabel amino acid incorporation into proteins from fresh brain tissue using short term tissue culture techniques; 7) Autofluorography of radiolabeled proteins; 8) Subcellular fractionation of central nervous system tissue.

Major Findings:

(A) The role that norepinephrine plays in regulating the concentration of different proteins in the parietal cortex, hippocampus and cerebellum was assessed by investigating the effects of either a bilateral lesion of the locus coeruleus or neonatal administration of 6-hydroxydopamine. Two weeks after lesioning the locus coeruleus, the concentration of two different proteins was elevated in the hippocampus; a third protein was reduced in concentration in this brain area as a result of the lesion. Three proteins were affected in concentration in the cerebellum after the locus coeruleus lesion - two were elevated in concentration and one was reduced in concentration. No proteins were altered in concentration in the parietal cortex as a result of the lesion. Seventy days after neonatal treatment with 6-hydroxydopamine, a total of six proteins were found to be changed. Four of these (one in the hippocampus and three in the parietal cortex) were reduced in concentration while two proteins (both in the cerebellum) were elevated in concentration after neonatal treatment with the catecholamine neurotoxin. There was little overlap between those proteins affected in concentration by the bilateral lesion of the locus coeruleus and those changed by neonatal treatment with 6-hydroxydopamine. These results suggest that the concentration of a number of different proteins may, under normal physiological conditions, be regulated *in vivo* by norepinephrine in the brain.

(B) The effect of chronic administration of clorgyline, a type A monoamine oxidase inhibitor, on the relative concentration of proteins from rat brain was examined by analysis of two-dimensional electrophoresis gels. The results from this study show that the administration of clorgyline for three weeks produced a significant elevation in the relative concentration of two proteins in the parietal cortex (MW 23,000 and 30,000) and one protein in the hippocampus (MW 25,000). In contrast, the relative concentration of three proteins (MW 31,000, 42,000 and 45,000) was significantly reduced by chronic clorgyline treatment. Since a previous study has indicated that the relative concentration of three different proteins are significantly altered by the repeated administration of desipramine, the results from this experiment indicate that different protein changes are produced by repeated treatment with the type A monoamine oxidase inhibitor clorgyline as compared to those produced by the tricyclic antidepressant desipramine. These results are consistent with the idea that different classes of antidepressant compounds alleviate depression through different mechanisms of action.

(C) The subcellular location of individual proteins visible on two-dimension gels of rat cortex generated using crude tissue punches was determined via subcellular fractionation combined with two-dimensional gel electrophoresis and computerized scanning densitometry. A total of six rat brains were homogenized in a buffered sucrose solution. This crude homogenate was then fractionated into seven different subcellular components: crude synaptic vessicles, cytosol, microsomes, mitochondria, myelin, nucleus and synaptic membranes. The proteins contained within 50 μg samples of each fraction were then separated by two-dimensional gel electrophoresis, stained with silver and analyzed by computerized scanning densitometry. Since the fractionation procedure produces enriched fractions but not a total separation of individual subcellular components, most proteins appeared in multiple fractions. Consequently, we selected a concentration factor of 1.5 as the minimum criteria for saying that an individual protein was present in one particular fraction. The concentration factor is defined as follows:

$$\frac{\text{optical density value in fraction containing the largest amount of protein X}}{\text{optical density value in fraction containing the 2nd largest amount of protein X}}$$

Using this as a criteria, of the 115 proteins analyzed, 61 (53%) were determined to be present primarily in a single fraction. The breakdown by fraction is as follows:

Cytosol - 20 proteins (17%)
Mitochondria - 16 proteins (14%)
Microsomes - 9 proteins (8%)
Nucleus - 9 proteins (8%)
Crude synaptic vessicles - 5 proteins (4%)
Myelin - 1 protein (<1%)
Synaptic membranes - 1 protein (<1%)

This identification of the subcellular localization of individual proteins will prove valuable in efforts aimed at determining the function of each protein in brain tissue.

(D) The effect of salt loading and water deprivation on the relative rate of turnover of individual proteins within the subfornical organ was investigated. For four days, three groups of rats (control, 2% NaCl, and water deprived) were given an appropriate fluid diet, with all groups having free access to food. Rats were killed by decapitation, the subfornical organ was quickly dissected out and incubated for 6 hours in a medium containing ^{35}S-methionine and ^{35}S-cysteine. The proteins from these organs were then separated by two-dimensional electrophoresis. The gels were then stained, photographed, dried and exposed to autoradiographic film. The results show that the relative turnover rate of eight proteins were changed due to the experimental manipulations. Four of these proteins are apparently sensitive to both salt loading and water deprivation, as both experimental manipulations caused a change in the relative rate of protein turnover. Of the other four proteins, three were affected by water deprivation while a single protein was sensitive to only salt loading. These results represent the first biochemical study of the subfornical organ in vitro, and provide information as to the biochemical effects of changes in fluid homeostasis on the subfornical organ.

(E) Two-dimensional gel electrophoresis with silver staining was used to study protein patterns in 20 high-grade human astrocytomas (anaplastic astrocytomas and glioblastomas) obtained during surgery. Histological correlates of the sampled tissue were carefully established. There was a general consistency in the protein pattern from one sample to the next, despite variations in certain spot densities. When these patterns were compared to those of normal human cerebral cortex, several proteins (most notably albumin and glial fibrillary acidic protein) were clearly more prominent in the tumor gels, while others were comparatively diminished. Several of the prominent protein spots including albumin, actin, alpha and beta-tubulin, neuron specific enolase, glial fibrillary acidic protein and glutamic oxaloacetic transaminase have been identified. A major strength of this technique lies in its capacity to semi-quantitatively display a large number of proteins simultaneously, using just a few milligrams of tissue. Its potential applications to diagnosis and to the study of tumor biology are under investigtion.

(F) Using two-dimensional gel electrophoresis, an apparent genetic polymorphism was detected in the hypothalamus of a group of inbred Sprague-Dawley rats. The proteins involved in this polymorphism have a molecular weight of 57,000 daltons and isoelectric points ranging from 6.1 to 6.3. These proteins met four criteria that should be met before a positional shift on two-dimension gels can be attributed to a genetic polymorphism. This is the first report of the existence of a genetic polymorphism in the brains of a group of inbred Sprague-Dawley rats. The functional significance of this polymorphism is currently under investigation.

(G) Using a sensitive and specific antiserum, the existence of two subforms of soluble glutamic oxaloacetic transaminase in both rat and human brain has been demonstrated. In the rat, the two proteins each have a molecular mass of 47,000 daltons. The more abundant basic protein has an isoelectric point of 5.9 while the sparsely staining more acidic protein has an isoelectric point of 5.8. In the human the two proteins visualized each have a molecular mass of 43,000 daltons. The more abundant basic protein has an isoelectric point of 5.7 while the sparsely staining more acidic protein has an isoelectric point of

5.6. In both rat and human, it seems reasonable to conclude that the abundent basic protein that reacts with the antiserum is the α subform of the enzyme, while the sparsely staining more acidic protein is the β subform of the enzyme. These results are of interest for a number of reasons. First, they establish the existence of at least two subforms of soluble glutamic oxaloacetic transaminase in both rat and human brain. Secondly, they show that this enzyme is a major protein visible on two-dimension gels of rat and human brain, and that this enzyme is widely distributed throughout the central nervous system. Finally, they positively identify two more proteins visible on two-dimension gels generated using rat and human brain tissue.

Significance to Biomedical Research and the Program of the Institute: The demonstration that specific proteins within the hippocampus, parietal cortex and cerebellum are regulated in concentration by norepinephrine suggests that these proteins may play a role in neuropsychiatric conditions (such as depression) whose biochemical basis is believed to be related to alteration in catecholamines. Likewise, the finding that specific proteins within the hippocampus and parietal cortex are altered in concentration by chronic treatment with the type A monoamine oxidase inhibitor clorgyline indicates a possible role for these proteins in depression. The fact that the tricyclic antidepressant desipramine and the monoamine oxidase inhibitor clorgyline have different effects on central nervous system proteins supports the theory that there may not be a common mechanism for these two classes of compounds in the treatment of depression. This suggests that patients refractory to one class of antidepressants may respond when another class of compounds is tried. The determination of the subcellular location of proteins visible on two-dimension gels generated using whole brain will provide information as to the identity of unknown proteins. The identification of a subset of proteins within the subfornical organ that show an altered rate of turnover after either salt loading or water deprivation suggests that these proteins may play a role in the normal maintenance of fluid homeostasis. Further characterizations of the two-dimension gel pattern of proteins present within astrocytomas, and the demonstration that these patterns are different from those generated in normal human cortex opens up the possibility of developing tumor-specific therapeutic agents that will increase the likelihood of eradicating the tumor with minimal damage to normal tissue. In addition, the specific protein patterns seen in astrocytomas may prove useful as an adjunct in the diagnostic process. The identification of a genetic polymorphism in the brains of a group of inbred Sprague-Dawley animals opens up the possibility of uncovering the biochemical basis for various inbred functionally abnormal strains of rats (e.g., Zuker Obese rats, Brattleboro hypertensive rats etc.). In addition, such a finding suggests that animals bred for a specific neurogenetic makeup may become available, making it possible to study the function of individual brain proteins while reducing interindividual variability. Finally, the identification of the isozymes of soluble glutamic oxaloacetic transaminase on two-dimension gels of rat and human brain demonstrates that this enzyme is one of the most abundant proteins in brain. In addition, this work shows that, immunologically, there are only two isozymes of serum glutamic oxaloacetic transaminase in brain, a situation that contrasts with the three forms of this enzyme present in most peripheral tissues.

Proposed Course of the Project:

1) Continue studies on proteins in brain which are regulated by specific neurotransmitters. We plan to investigate the effect of serotonin depletion on the concentration of specific proteins in different cortical areas of the central nervous system.

2) Begin examining the effect of depletion of specific neurotransmitters on the relative rate of turnover of specific proteins in the brain *in vivo*.

3) Continue studies aimed at further characterizing proteins in both malignant and benign human brain tumors.

4) Identification of proteins on two-dimension gels using a combination of co-migration with purified protein standards and cross-reactivity with specific antisera.

5) Further investigation of radiolabeled amino acid incorporation into protein *in vitro*, focusing on those areas of the central nervous system that contain the cell bodies for norepinephrine, serotonin and acetylcholine.

6) Identification of the subset of proteins present on two-dimension gels of rat brain that are brain specific.

7) Isolation and characterization of proteins that the above experiments reveal to be of possible interest.

Publications:

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Gold, M. A., Heydorn, W. E., Creed, G. J., Weller, J. L., Klein, D. M. and Jacobowitz, D. M.: *In vitro* [^{35}S]-methionine labeled protein synthesis in microdissected discrete brain areas: Marked regional differences revealed by two-dimensional gel electrophoresis. *Electrophoresis* 5: 116-121, 1984.

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Heydorn, W. E., Creed, G. J., Wada, H. and Jacobowitz, D. M.: Immunological evidence for the existence of two subforms of soluble glutamic oxaloacetic transaminase (sGOT) in human and rat brain. Neurochemistry International (in press).

Narayan, R. K., Heydorn, W. E., Creed, G. J., Kornblith, P. L. and Jacobowitz, D. M.: Two-dimensional gel electrophoretic protein patterns in high grade human astrocytomas. J. Neuro-Oncology (in press).

Sills, M. A., Heydorn, W. E., Cohen, R. M., Creed, G. J. and Jacobowitz, D. M. Effect of Chronic Chlorgyline Treatment on the Relative Concentration of Specific Proteins in Rat Hippocampus and Parietal Cortex. Neuropharmacology (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00397-07 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurophysiological Effects of Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Matthew A. Sills	Guest Researcher (NIGMS Fellow)	NIGMS & LCS, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recently, increased attention has focused on a new class of peptides, termed atrial natriuretic factor(s), or ANF, which was initially found in atrial tissue extracts. When administered peripherally, potent natriuretic and diuretic effects on the kidney have been produced by this peptide. Histological studies in rat brain have revealed recently that a dense localization of ANF-containing neurons exist in the anteroventral third ventricle, or AV3V area of the brain. Based on these findings, ANF was microinjected into this region of the brain. Blood pressure (BP) and heart rate (HR) responses were monitored to study the physiological significance of the localization of ANF in this region of the brain. The results from this study reveal that pmol quantities of ANF produce substantial increases in both BP and HR. Mean arterial, systolic and pulse pressures as well as HR were significantly elevated by ANF in comparison to the effect produced by a prior injection of saline. Systolic pressure, pulse pressure and HR were found to increase in a dose-dependant manner. The onset of these cardiovascular effects was seen 15-45 min after injection. Peak effects were usually observed approximately 90-120 min after onset, and the duration of these effects was 3-4 h. The results from this study provide evidence for a functional role of ANF in the AV3V area of the brain, and lend support to the idea that ANF may play an important role in central cardiovascular regulatory mechanisms.

Project Description

Objectives: The major goal of this study was to establish a functional correlate for the dense localization of ANF-containing neurons in the AV3V area of the brain. Since this brain region has been shown to be closely involved in blood pressure regulation, as well as fluid and electrolyte balance, the effect of ANF microinjected into the preoptic suprachiasmatic nucleus (posc), which is located in the AV3V region, on BP and HR were examined.

Methods Employed: BP and HR measurements were determined by inserting a cannula, connected through a transducer to a Biograph recorder, into the femoral artery of anesthetized rats. Nanoliter quantities of ANF were injected into the posc of the rat using double-barreled glass micropipettes. Injection sites were determined by histological examination of serial, frontal cryostat sections stained with thionin.

Major Findings:

(A) Injections of 2,4,20 or 40 pmol quantities of ANF into the posc produced significant increases in mean arterial, systolic and pulse pressures in comparison to prior injections of saline. The effects elicited by ANF on systolic and pulse pressures increased in a dose-dependant manner. Although all four doses of ANF produced an increase in diastolic pressure, only the 20 pmol dose produced a significant elevation in comparison to saline.

(B) ANF injections elicited a significant increase in HR as compared to prior injections of saline. A dose-dependant elevation of HR was observed. Lower doses of ANF (2-4 pmol) produced a modest increase in HR (approximately 5-10%) whereas the higher doses of ANF (20-40 pmol) elevated HR by approximately 20%.

(C) The onset of effects on BP and HR produced by ANF was seen 15-45 min after injection. Peak effects usually occurred 90-120 min after onset, and the duration of the effects was 3-4 h, after which time values usually returned to baseline.

(D) The results from this study indicate that: (a) in contrast to the depressor effects produced by ANF when administered peripherally, a rise in both BP and HR were observed subsequent to injections into the posc; (b) the ability of ANF to increase pulse pressure in addition to mean arterial pressure indicates that ANF produces a greater effect on cardiac output than on peripheral resistance; (c) the delayed onset and long lasting effects produced by ANF indicates that the mechanism by which these effects are produced may involve either a breakdown product of ANF or that ANF-induced protein synthesis.

Significance to Biomedical Research and the Program of the Institute: Our laboratory has been involved with elucidating the histological localization of peptides within the brain. In concert with these studies, we have attempted to establish functional correlates for these compounds in specific

areas of the brain. The finding that pmol quantities of ANF produced substantial changes when injected into the posc, an area found to contain a dense localization of ANF-containing neurons, provides evidence for a possible functional and/or physiolgical role of ANF in central cardiovascular regulation. This initial study in conjunction with future studies planned to examine the mechanism by which ANF produces its effects are necessary to provide an understanding of the role of neuropeptides in the brain. With this knowledge, a better understanding of the consequences of deficits in neuropeptide systems will be achieved.

Proposed Course of the Project: 1) To examine whether the effects produced by ANF are mediated by protein synthesis or by a metabolite, studies will examine whether protein synthesis inhibitors are able to block the effects of ANF, and whether peptide fragments of ANF produce similar responses. 2) To determine whether ANF produces its effects directly or by modulating other neurotransmitter systems, a pharmacological series of antagonist compounds will be examined for their ability to block the effects of ANF.

Publications:

Diz, D. I. and Jacobowitz, D. M.: Cardiovascular effects of discrete intra-hypothalamic and preoptic injections of bradykinin. Brain Res. Bull., 12: 409-417, 1984.

Diz, D. I. and Jacobowitz, D. M.: Cardiovascular effects produced by injections of thyrotropin-releasing hormone in specific preoptic and hypothalamic nuclei in the rat. Peptides 5: 801-808, 1984

Diz, D. I. and Jacobowitz, D. M.: Cardiovascular actions of four neuropeptides in the rat hypothalamus. Clin. Exp. Hypertension, A6: 2095-2090, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00820-02 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behaviors of Rats Associated with Their Vocalizations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John B. Calhoun Chief URBS LCS NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated as a discrete research project. It has been integrated into Z01 MH 02239-01, which reports on progress toward preparation of a book length publication integrating the results of several projects conducted from 1974 to 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00849-03 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Resting Time Residence in a 7-Generation Population of House Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John B. Calhoun Chief URBS LCS NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated as a discrete research project. It has been integrated into Z01 MH 02239-01, which reports on progress toward preparation of a book-length publication integrating the results of several projects conducted from 1974 to 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00850-03 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cooperation Induced Modification of Behavior in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John B. Calhoun Chief URBS LCS NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated as a discrete research project. It has been integrated into Z01 MH 02239-01, which reports on progress toward preparation of a book length publication integrating the results of several projects conducted from 1974 to 1983.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02239-01 LCS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conceptual Analysis of Complex Biobehavioral Population Systems.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John B. Calhoun Chief URBS LCS NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	1.00	2.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The present project concerns research strategies of analysis and conceptual integration of three large scale interrelated research projects conducted between 1974 and 1984. Two of these deal with rodent populations subjected to increase in population density over successive generations. The third concerns the interrelationship between the evolution of human populations and the evolution of knowledge. Theory developed from the latter effort influenced the design of the rodent studies. These studies with rodents are intended to serve as animal models for how health and well-being of humans are influenced by living in complex physical and social environments. Therefore, the settings and time course to which rodent subjects were exposed similarly challenged them to adjust to progressively increasing complexities. Large data bases, involving many variables, were developed. Analyses of each variable leads to first order conclusions and restructuring the data bases into new kinds of data. These permit second order analyses and conclusions. Gradually different kinds of second and third order analyses are brought into interrelationship to form even higher order integrative analyses. Such strategy gradually culminates in a relatively small set of principles of process. For example, analyses of places of residence of each subject enabled categorization of life course into episodes of residential stability or instability. Across generations, residential stability of the population declined exponentially. Similarly, analyses of age at death revealed that mortality rate of females exceeded that for males, and that this difference between sexes increased with crowding as more females developed mammary tumors. Further, examination of residential stability revealed that after inception of crowding, rate of increase in mortality with age increased much faster among female mice.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: To develop methods of inquiry, data analysis, concept integration and written communication which will make the value or impact on science and society of each cycle of inquiry nearly equal to the sum of the impact of all prior cycles. Impact, for practical purposes, is here construed to mean number of citations to our work in Citation Index. The number of citations to my research has doubled each 4.15 years. This means that there have been 5.45 times as many citations to my work each 10 years as were made the prior ten years. 1984 marked the 4th 10-year cycle of my research since obtaining my doctorate in 1943.

Results of each of my prior 10-year cycles of research resulted in approximately 500 printed pages. That is to say, volume of publications per unit time is a constant. Since the number of citations increases 5.45 fold each 10 years, it follows that a page of publications during one cycle, on the average, carried 5.45 times as much meaningful information as those resulting from the prior cycle. Such increase in information content results partly from experience enhanced ability to design better animal model studies that simulate processes whereby human health and well-being are influenced by living within complex physical and social environments. However, the issue of particular concern is how to make more effective our developing strategies of iterative integration of separate analyses, until a few basic principles are clarified that will make more understandable the status of the individual in large, "N-body", interactive systems. Empirically this will be attempted in a 500 page space allotted us for this purpose by the Johns Hopkins University Press for publication in book form. It will consist of circa 125 1800-word space sections, each equivalent to a short scientific paper that might have been published separately in a scientific journal, but will be much more effective within an integrated collection.

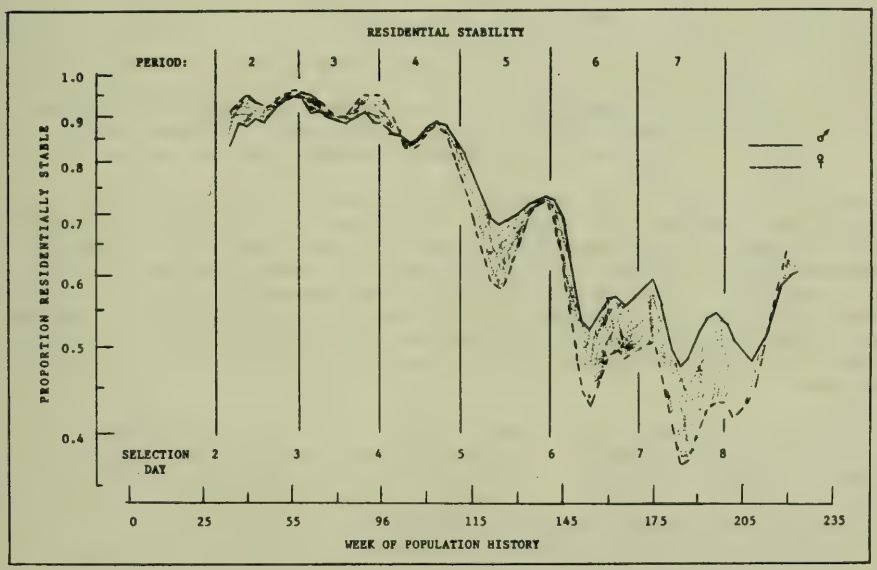
Methods:

The particular animal model studies involved are not of concern here, but rather our focus is on how items of data about known individual members are analyzed and integrated to provide an understanding of multi-individual biobehavioral social systems. An essential question concerns the adequacy of the data base for this purpose. Our realized intent was to record a large sample of events or conditions relating to health and behavior of identified individuals with time (date and time of day) and place of occurrence associated with each such element of data recorded. 10 million such data items (representing over 200 major kinds of items) were scattered across the lifetimes of 2500 subjects. On the average, approximately 4,000 data items were recorded for each subject. We suspect that this is just above the minimum requisite to explore the interaction among processes characterizing such complex behavioral systems as we have investigated.

Major Findings:

"Findings" in the present project are represented by strategies of analysis and integration which enable condensation of the relationships among several different conclusions into a single unified formulation of a principle about system's process. An example from one of the animal model studies will partially clarify the nature of the overall strategy.

Figure 1 represents the change of residential stability of male and female adult mice in Study 133. At the end of each sequential ca. 200-day Period, enough young were allowed to survive so that they, added to the surviving adults, would double the population. Population density remained within the optimum range during Periods 2 and 3. From Period 3 through 7 the proportion of mice maintaining stable places of residence declined exponentially as the adult population progressively attained densities of 2, 4 and 8 times the optimum. During each period the minima of residential stability are associated with maximum conflict between younger maturing mice and older ones for a limited resource, social roles. Likewise, maxima are associated with the social stabilization of relations after the replacement of many older mice by younger ones has been completed, and these replaced older mice have withdrawn into states of reduced activity and social interaction. Note that females progressively become more unstable residentially than males as crowding increases. Had we not simultaneously been conducting analyses of health and mortality we might well have not made this comparison of males and females with regard to residential stability.



Date of death relative to date of birth by subject identity and sex represents a basic element of data. Age at death is a first order derivative datum. Across subjects, the proportion of subjects which live to any age, A, but die before the end of a following constant interval, i, represents a second order derivative datum, mortality rate. Increase in mortality rate with age represents a third order datum which is also a conclusion. That the female mice were characterized by a more rapid increase in mortality rate, represents a fourth order datum/conclusion. This difference between sexes becomes more accentuated as crowding increases. At or about the time of death, including autopsies, various items

relating to health were recorded. Each formed a basic element of data. Prevalence of two such elements, enlarged mammary tumors and inability or difficulty in delivering near-term young, increased with crowding. Such mortality related factors suggested an examination of sexual differences in residential stability.

Figure 1 was the result. Annual Report No. Z01 00849-02 LBEB for 1984 describes how the basic element of data, place of capture at a time of day of reduced activity level, enables the fourth order datum/conclusion of the present Figure 1.

Significance to Biomedical Research and the Program of the Institute: Over the past 25 years considerable unresolved opinion, even acrimony, marks the differences between proponents of "reductionistic" versus "holistic" research. The former point to the rapid rate of progress among disciplines emphasizing reductionist methodology. The latter remark that reductionistic inquiry can never provide much useful insight into how the health and well-being of an individual is influenced by living within complex physical and social behavioral settings. A recent reviewer in the New York Book Review (Slobodkin) lays the onus on behavioral scientists for not producing those principles of process which characterize larger aggregates of life. This reviewer is certainly correct, but mere statement of the proposition remains vapid without facing the problem of the increasing difficulty of conceptualizing process as higher levels of organization of nested systems within systems are confronted. As higher levels of organization of living matter are approached, the number of variables increase and system complexity and duration of process change increase proportional to the square of the number of variables characterizing the system.

We have tried to resolve this dilemma of conducting profitably holistic research on complex systems. It is our conclusion that serendipity and creative conceptualizations can be optimized by (a) simultaneously conducting at least three somewhat related complex, large-scale, long-term research projects; (b) on any one, or across the three research projects, simultaneously pursuing at least three possibly related avenues of analysis and integration; (c) utilizing computer facilities to enhance creative conceptualizations by simulating brain functions in ways that partially overcome the limited channel and associative capacities of the human brain.

The ultimate definition of mental health involves ability of the individual to adapt satisfactorily to changes in the experienced complexities of the physical, social, and conceptual environments. If the NIMH is interested in mental health, so defined, then the trust placed in it will bear fruit proportional to the effort it supports to further understand how more effectively to engender comprehensive, unifying, conceptualizations of processes ongoing in complex systems. We are indebted to the many years of support by the NIMH which has enabled us to make some progress on related animal models, as well as on strategy of research conduct, and of analysis and integration of ideas.

Future Course: Complete the writing of the 125 sections which will comprise the presentation of the 1974-1984 research effort by the Unit for Research on Behavioral Systems.

Understanding what the impact of the proposed course may be requires consideration of the long delay characterizing citations to literature analyzing complex living systems. After cessation of writing for publication in order to

conduct the 1974-84 research, there were during these eleven years 1441 citations to my 1941-73 publications. These 1441 citations are 2.8 times the 369 to my work between 1941-1973. One would anticipate in excess of 3500 citations in Citation Index to the presently being prepared book after it is published by the Johns Hopkins University Press.

Publication:

Calhoun, John B.: The transitional phase in knowledge evolution. Man-Environment Systems 14: 131-142, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00153-08 CHP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Obsessional Children and Adolescents with Chlorimipramine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith L. Rapoport, M. D., Chief, Child Psychiatry Branch, NIMH
David Shaffer, M. D., Columbia University
Martine Flament, M. D., Guest Researcher, CHP, NIMH
Dennis L. Murphy, M. D., Chief, LCS, NIMH
Theodore Zahn, Ph.D., Research Psychologist, LPP, NIMH
Agnes Whittaker, M. D., Columbia University
Paul Fedio, Ph.D., Acting Chief, CN, NINCDS
Martha Denckla, M. D., Chief, Autism and Behavioral Disorders Section, DNB, NINCDS

COOPERATING UNITS *(if any)*

Unit on Sleep Studies, CPB, NIMH; Laboratory of Psychology and Psychopathology, NIMH; Clinical Neuropharmacology Branch, NIMH; National Institute of Neurological and Communicative Disorders and Stroke; Columbia University

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	.75	.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Obsessional disorder of childhood is a severe psychiatric disturbance which is being increasingly recognized and treated. To date, a double-blind crossover trial of clomipramine has been shown effective in the treatment of this disorder.

Clinical improvement does not correlate with plasma concentration of the drug, but does correlate significantly with decrease in platelet serotonin.

A follow-up study of the 30 subjects who have, to date, participated in in the drug trial is underway. Preliminary data indicate that 50% of the group are improved; the rest remain moderately to severely impaired.

An epidemiological survey has just been completed of over 5000 high school students, in which obsessive compulsive symptomatology was rated. Follow-up interviews of high scorers on this questionnaire indicate that .3% of the adolescent population may have some form of this disorder, a rate far in excess of previous estimates.

Project Description:

Objectives: To examine the natural history, neuropsychological and neuroradiological measures in obsessive compulsive children at follow-up. This is to complement the preliminary studies which indicated subtle differences between patients and controls on some neuropsychological psycholinguistic tasks, as well as increased ventricular brain ratios for the obsessive adolescents CT scans, relative to normal controls.

Methods Employed: Patients participating in the study of children and adolescents with pure primary obsessive compulsive disorder between 1976 and the present are being seen for follow-up evaluation. At this time, structured psychiatric interview, clinical history of patient and family are obtained. In addition, neuropsychological and psycholinguistic testing, CT scan are being repeated, as well as, when available, Positron Emission Tomography. The dexamethasone supression test is also repeated.

As part of the initial drug trial, a self-report questionnaire was developed, for obsessive and compulsive symptomatology. In collaboration with Drs. David Shaffer and Agnes Whittaker, Columbia University, this questionnaire was used in an epidemiological survey of the 5500 high school students in Warren County, New Jersey. "High scorers", representing the highest 1% on the questionnaire were then interviewed by Branch clinicians with modified versions of the SADS and DICA, structured psychiatric interviews, for depressive, anxiety, and eating disorders as well as obsessive compulsive disorder.

Major Findings: Preliminary follow-up of the eight obsessive patients seen to date indicates continued severe psychopathology in half of the cases. Even those functioning well, have, with one exception, occasional symptoms. One case developed a psychotic disorder; for all the other cases the original diagnosis would still apply. The most common secondary diagnoses were anxiety disorder or depressive disorder. While 12 children were discharged on maintenance clomipramine, only three appear to have continued on the drug long-term. Three adolescents made impulsive suicidal gestures with medication, all, while on maintenance drug treatment.

The epidemiological survey was followed up by a direct interview of 65 adolescents from six different schools. Twelve of these subjects were diagnosed as having obsessive compulsive disorder (mild to moderate) - none were from the normal control group or from the group of "psychiatric controls". This represents a most conservative estimate of frequency of this disorder as the screening questionnaire would only identified 50% of our clinical cases. Furthermore, those subjects with very severe forms of the disorder would not be attending school. The probable incidence of the disorder, therefore, is at least .30% of the adolescent population. In addition, a number of adolescents appeared to have a subclinical entity with sudden onset that was best described as "obsessive features". This did not appear to be continuous with obsessive personality disorder, and is of uncertain clinical significance. In some cases, it appeared

to be a variety of post-traumatic stress disorder, for others it may be a very early form of Obsessive Compulsive Disorder.

Significance to Mental Health Research: The follow-up study indicates that children and adolescents with the disorder are at risk for continuing severe psychopathology. The epidemiological study addresses the notion of continuity and discontinuity in developmentand the prevalence of the disorder. The availability of a pharmacological agent, together with other behavioral treatments developed elsewhere are important advances for a disorder with notorious treatment resistance.

Proposed Course of Project: The clomipramine trial will be extended for 20 additional adolescent patients to increase sample size as well as to see if a non-crossover random assignment drug trial will reach statistical significance. New subjects must first have an adequate trial of another tricyclic antidepressant before entering the trial. There will be a follow-up of the subjects identified in the epidemiological study as having mild disorder and "obsessive features" to see whether these are early states of the disorder or represent different entities.

Publications:

Rapoport, J.: Obsessive Compulsive Disorder. In Shaffer, D., Ehrhardt, A., and Greenhill, L. (Eds.): Diagnosis and Treatment in Pediatric Psychiatry. New York, McMillan, 1984, pp. 208-217.

Berg, C., Behar, D., Zahn, T., and Rapoport, J.: Obsessive Compulsive Disorder-An Anxiety Syndrome? In Gittelman, R. (Ed.): Anxiety Disorders. Guilford Press, New York, in press.

Flament, M., Rapoport, J., and Berg, C.: Childhood Obsessive Compulsive Disorder. In Insel, T. (Ed.): Obsessive Compulsive Disorder. APA Press, 1984, pp. 23-44.

Flament, M., Rapoport, J., Berg, C., Sceery, W., Kilts, C., Mellstrom, B., Linnoila, M.: Clomipramine treatment of children with obsessive compulsive disorder: A double blind controlled trial. Arch. Gen. Psychiatry, in press.

Berg, C., Rapoport, J., Flament, M.: The Leyton Obsessional Inventory - Child Version. J. Amer. Acad. Child Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00161-07 CHP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Behavioral Effects of Dietary Substances in Normal and Hyperactive Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Markus Kruesi, M. D., Clinical Associate, CHP, NIMH
Martine Flament, M.D., Guest Researcher, CHP, NIMH
Mark Cummings, Ph.D., LDP, NIMH
Marion Yarrow, Ph.D., LDP, NIMH
Carolyn Zahn-Waxler, M. D., LDP, NIMH
Thomas Uhde, M.D., BPB, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Developmental Psychology, NIMH
Biological Psychiatry Branch, NIMH

LAB/BRANCH

SECTION
Child Psychiatry Branch, NIMH

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	.75	.75

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of glucose, sucrose (1.75gm/kg), aspartame (30mg/kg) and saccharin were compared for 32 preschool aged males: 19 had histories of adverse behavioral response to sugar while 13 were familiar playmates with no such histories. There was no evidence of behavioral response to sugar on measures of activity, aggression, expression emotionality or anxiety. There was a slight but significant decrease in actometer rated motor activity following aspartame ingestion, the significance of which is unclear.

The response of children with generalized anxiety disorder and normal controls to caffeine (10 mg/kg) are being compared. To date, a total of four pairs have been examined with no evidence for greater responsivity of anxiety disordered children that controls. This is in contrast to studies with adult patients indicating caffeine hypersensitivity.

Project Description:

Objectives: The relationship between dietary substances and behavior in normal and disturbed children are being investigated in an ongoing series of studies; responses to caffeine, sugar, and aspartame are being explored.

Methods Employed: For the sugar-aspartame study, 32 children (19 with alleged sugar reactivity and 13 familiar playmate companions with no known history of dietary responsivity) were studied following challenges of aspartame (30mg/kg), glucose, sucrose (1.75gm/kg) or saccharin. Children were also challenged in their home setting with only a parent as an observer also utilizing a random order, double blind design. Dependent measures were actometer rated activity, behavioral rating scales and for the NIH session, taped play sessions following structured and unstructured periods.

For the caffeine study, the response of four pairs of children (one having generalized anxiety disorder and one age matched control) was examined following challenges of dextrose and quinine placebo, 3 and 10 mg/kg. Dependent measures were anxiety ratings, autonomic ratings, salivary cortisol and caffeine.

Major Findings: There was no difference on any measure for any of the children's response to saccharin, glucose or sucrose at home or at the NIH. In general, this was also true for aspartame; however, there was significant decrease in actometer measured motor activity during the two hours following aspartame ingestion. The significance of this is unclear, but does not appear to be clinically interesting as no observer rated behavior changed significantly following aspartame.

Preliminary data from the four pairs of children who received caffeine challenges do not indicate a differential sensitivity to caffeine for the anxiety disordered children and controls.

Significance to Mental Health Research: There is interest in the possibility that aspartame might produce adverse responses in children either by increasing brain phenylalanine or through some unknown mechanism. This is the first controlled trial to study this both in normal and hyperactive children. In addition, sugar ingestion has been correlated with inattentive, restless behavior in a large group of preschool children. The present study is the first challenge study in this age group.

The response of prepubertal anxiety disordered children to caffeine appears different than that of older subjects with anxiety disorders who have been shown more responsive to caffeine than are controls. This finding substantiated in the remainder of the study suggests that prepubertal anxiety disordered children are physiologically different from post pubertal patients with perhaps less sensitive noradrenergic response to caffeine. This has implications for biological risk research for anxiety disorders.

Proposed Course of Project: No further studies of sugar or aspartame sensitivity are planned. The caffeine challenge study will be extended and further studies will depend upon the results of this trial.

Publications:

Rapoport, J., Berg., C., Ismond, D., Zahn, T., and Neims, A.: Behavioral effects of caffeine in children: Relationship between dietary choice and effects of caffeine challenge. Arch. Gen. Psychiatry, 41: 1073-1077, 1984.

Kruesi, M.J.P., Linnoila, M., Rapoport, J.L., Brown, G.L., Petersen, R.D.: Carbohydrate craving, conduct disorder and low 5-HIAAA. Psychiatry Res., in press.

Rapoport, J.L., Kruesi, M.J.P.: Behavior and nutrition: A mini review. ASDC J. Dent. Child. 51: 6, 451-454, 1984.

Rapoport, J.L., Kruesi, M.J.P.: Diet and childhood behavior. In Office of the Surgeon General: Report on Nutrition and Health, in press.

Kruesi, M.J.P., Rapoport, J.L.: Diet and human behavior: Do they impact upon each other; if so, how much? Annu. Rev. Nutr., in press.

Kruesi, M.J.P., Rapoport, J.L.: Dietary constituents and behavior. Trends in Neurosciences, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00162-06 CHP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Hyperactive Children with Desmethylimipramine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith L. Rapoport, M. D., Chief, CHP, NIMH
Maureen Donnelly, M. D., Clinical Associate, CHP, NIMH
Alan Zametkin, M. D., Clinical Associate, CHP, NIMH
Willliam Z. Potter, M. D., Ph.D., Chief, Section on Clinical Pharmacology, LCS, NIMH
Herbert Weingartner, Ph.D., Psychologist, LPP, NIMH
Markku Linnoila, M. D., Ph.D., Chief, LCS, NIAAA

COOPERATING UNITS *(if any)*

Laboratory of Psychology and Psychopathology, NIMH
Laboratory of Clinical Science, NIMH
Laboratory of Clinical Studies, NIAAA

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A controlled treatment trial of desmethylimipramine (DMI) (up to 3.4 mg/kg/day) or placebo was completed with 29 hyperactive boys. There was significant and immediate (within three days) efficacy of DMI, sustained for the two week duration of the study. Plasma and urinary MHPG excretion was significantly decreased at days three and 14 and this decrease correlated with behavioral improvement. The study supports a role for norepinephrine in mediating the efficacy of stimulant medications in the treatment of Attention Deficit Disorder (ADD).

Project Description:

Objectives: To examine the clinical effects of a relatively specific tricyclic antidepressant in relation to plasma and urinary indices of norepinephrine (NE) metabolism.

Methods Employed: DMI (up to 3.4 mg/kg/day) or placebo were administered for two weeks to a total of 29 hyperactive boys. Nineteen received DMI and 12 received placebo using a double blind, non-crossover design. Home and classroom behavior as well as vigilance on a monitoring task, were monitored weekly. Plasma tricyclic concentration and urinary and plasma catecholamines were measured at days three and 14.

Major Findings: DMI has a significant anti-hyperactive effect which was apparant by day three. Unlike stimulants however, no benefit was observed on the Continuous Performance Test. Urinary and plasma MHPG were decreased at day three and at day 14. The plasma tricyclic concentration was low compared to antidepressant levels; mean values for DMI plus Oh-DMI were 33.7 mg/m. and 134 mg/ml at days three and 14 respectively. Behavioral improvement did not correlate significantly with plasma drug concentration at either timepoint. There was a significant decrease in urinary and plasma MHPG at days three and 14, which did correlate significantly with teacher rated behavioral improvement.

Significance for Mental Health Research: Hyperactivity is now recognized to be a significant disorder with lasting psychopathology. At least one third of hyperactive children have sustained difficulties with impulsive control disorders of adult life including: Antisocial personality disorder, gambling disorder, alcohol and drug abuse. Understanding the mechanism of hyperactivity could lead to more effective preventive treatment with possible prevention of these disorders later in life.

Projected Course of Project: This clinical trial of DMI has been completed. The research on the pathophysiology of ADD is ongoing and will be covered in the future under the more general heading of "Neurobiology of Attention Deficit Disorder".

Publications:

Donnelly, M., Rapoport, J.L.: Stimulant drug treatment of attention deficit disorder. In J. Wiener (Ed.): Diagnosis and Psychopharmacology of Childhood and Adolescent Disorders. New York, J. Wiley & Co., 1985, pp. 178-198.

Rapoport, J. L., Zametkin, A., Donnelly, M.: New drug trials for attention deficit disorder. Psychopharmacol. Bull. 21: 232-236, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00163-06 CHP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Naturalistic Study of Activity Levels of Hyperactive Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Linda Porrino, Ph.D., Guest Researcher, CHP, NIMH
Judith L. Rapoport, M. D., Chief, CHP, NIMH
Marcus Kruesi, M. D., Clinical Associate, CHP, NIMH
Thomas Wehr, M. D., Chief, CPB, NIMH

COOPERATING UNITS *(if any)*

Clinical Psychobiology Branch, NIMH

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated. Several talks have been given about this data and the NIH actometer is being made available to selected collaborators because of the positive response to this study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00165-05 CHP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological Markers of Alcoholism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith L. Rapoport, M. D., Chief, CHP, NIMH
Martine Flament, M. D., Guest Researcher, CHP, NIMH
Markku Linnoila, M. D., Ph.D., Chief, LCS, NIAAA
Anil Muhkerje, M. D., Ph.D., Chief, Section on Molecular and Developmental Genetics, PRB, NICHD
Irwin J. Kopin, M. D., Scientific Director, NINCDS

COOPERATING UNITS *(if any)*

Laboratory of Clinical Studies, NIAAA
Pregnancy Research Branch, NICHD
Office of Scientific Director, NINCDS

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.75	0.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated. Additional meetings were held with NIAAA to plan their more extensive high risk studies. Our subjects were contacted and made available to them.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00177-04 CHP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Hyperactive Children with Monoamine Oxidase Inhibitors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith L. Rapoport, M. D., Chief, CHP, NIMH
Alan Zametkin, M. D., Staff Psychiatrist, CHP, NIMH
Dennis Murphy, M. D., Chief, LCS, NIMH
Herbert Weingartner, Ph.D., Chief, Unit on Cognitive Studies, LPP, NIMH
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Farouk Karoum, Ph.D., NPB, NIMH
William Z. Potter, M.D., Ph.D., Chief, Section on Clinical Pharmacology, LCS, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Clinical Science, NIMH; Laboratory of Clinical Studies, NIAAA
Neuropsychiatry Branch, NIMH
Laboratory of Psychology and Psychopathology, NIMH

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.75	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thirty children with childhood Attention Deficit Disorder (ADD) have been treated with a selective or nonselective MAO inhibitor. The major findings to date are that both clorgyline, a selective MAO-A inhibitor, and tranylcypromine, a nonselective inhibitor, were effective in decreasing hyperactivity and improving attention. There was a significant decrease in urinary MHPG excretion which showed some correlation with behavioral improvement both on MAOIs and on d-amphetamine. However, this decrease in MHPG persisted for several weeks after drugs were stopped while ther was immediate behavioral rebound off drug for both amphetamine and for the MAOIs.

The study supports alteration of catecholamine metabolism as mediating the effects of stimulant medication. A controlled trial of l-deprenyl, a selective MAO-B inhibitor, is ongoing.

Project Description:

Objectives: Trials of relatively selective agents in the treatment of Childhood Attention Deficit Disorder with Hyperactivity (ADDH) have been disappointing compared with the dramatic efficacy of stimulant drugs. The purpose of these studies was to examine a class of agents known to effect catecholamine metabolism through another mechanism; in this case, by inhibiting the breakdown of dopamine and norepinephrine, as well as of serotonin. This is part of a series of studies on the pathophysiology of ADD.

Methods Employed: Hyperactive boys were treated with clorgyline (up to 15mg/day), tranylcypromine (10mg/day), or d-amphetamine (0.5mg/kg), using a double blind crossover design modified by a two week placebo washout period in between drug periods. Urinary and plasma catecholamines and metabolites, platelet MAO, and plasma 5 HT were measured to see whether these predicted or reflected drug effects.

After 14 subjects completed these first studies, 1-deprenyl, an MAO-B inhibitor (10 mg/day) was substituted, and a total of 18 children to date have completed this study.

Major Findings: While both clorgyline and tranylcypromine appear effective in the treatment of ADD, there is no close relationship between any alteration of urinary or plasma catecholamine or metabolite and clinical improvement on drug. The efficacy of cloryline supports a noradrenergic basis for this improvement. The immediate efficacy, and immediate relapse off drug, suggests a different mechanism from that in depression.

To date, 18 subjects have completed a trial with 1-deprenyl, a selective MAO-B inhibitor. Preliminary evidence suggests that this drug is less effective than either an MAO-A inhibitor or a mixed MAO-A & B inhibitor. Taken together, these findings also support noradrenergic mechanisms underlying the drug induced improvement of this disorder.

Significance to Mental Health Research: Hyperactivity is a risk factor for adult sociopathy, alcholism and possibly schizophrenia. Studies on the treatment and pathophysiology of hyperactive children have wide implications for prevention and treatment of these major conditions.

Proposed Course of Project: After completion of the low dose (10 mg/day) of 1-deprenyl, a second phase study of up to 30 mg/day as tolerated will be conducted. This will permit comparison of MAO-B inhibitors with MAO-A inhibitor (clorgyline) and a mixed inhibitor (tranylcypromine) for efficacy in the treatment of ADDH.

Publications:

Zametkin, A., Rapoport, J., Murphy, D., Linnoila, M., and Ismond, D.: Treatment of childhood attention deficit disorder with hyperactivity with monoamine oxidase inhibitors. Arch. Gen. Psychiatry, in press.

Zametkin, A., Rapoport, J., Murphy, D., Linnoila, M., Karoum, F., Potter, W., Ismond, D.: Urinary and plasma monoamines and metabolites in hyperactive children: Independence from behavioral state and persistence of changes post amphetamine. Arch. Gen. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00178-04 CHP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Structure and Function in Developmental Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith M. Rumsey, Ph.D., Staff Fellow, CHP, NIMH
Connie Duncan, Ph.D., Staff Fellow, LPP, NIMH
Richard Coppola, Ph.D., Engineer, LPP, NIMH
Stanley I. Rapoport, M. D., Chief, LN, NIA
Karen Berman, M.D., Staff Fellow, NPB, NIMH
Ronald Zec, Ph.D., Staff Fellow, NPB, NIMH
Daniel Weinberger, M. D., Chief, NPB, NIMH
Martha B. Denckla, M. D., Neurologist, NINCDS

COOPERATING UNITS *(if any)*

Laboratory of Psychology and Psychopathology, NIMH; Section on Autism DNB, NINCDS; Section on Brain Aging and Dementia, LN, NIA; Section on Clinical Neuropsychiatry, NPB, NIMH

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.0	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of adult males with severe developmental dyslexia are underway. A sample of 14 dyslexic men has been identified with a battery of academic and intelligence tests and interviews. Six matched normal controls have also been identified and others are being recruited. Subjects are being studied with EEG spectral analysis combined with topographic mapping and with xenon-inhalation procedures for the measurement of regional cerebral blood flow. Lateralizing tasks are being used in conjunction with these procedures. In addition, these subjects have also been characterized as dyslexic with or without attention deficit disorder and are being studied with an extensive battery of event-related potentials (ERPS) designed to differentially assess the ability to initiate, select, inhibit, shift, and sustain attention. Magnetic resonance imaging (MRI) is being used to examine brain anatomy. Other aspects include quantitative neurological examinations for soft signs and neuropsychological testing.

We anticipate completing these studies by early 1986 and proceeding to study subgroups of learning disabled children with many of the same methodologies. Thus, additional preparations (with respect to recruiting and test materials) are also underway.

Other projects include a series of studies of autistic adults. These include studies of brain anatomy with volumetric CT measures, studies of glucose metabolism with PET, autonomic nervous system studies, psychiatric, and neuropsychological studies. Additional PET-scans on autistic adults are still in progress and will provide a sample size adequate to apply intercorrelational analyses to examine metabolic patterns throughout the brain.

Project Description:

Objectives: These studies have as their goal the identification of neuroanatomical, neurophysiological, and neuropsychological deficits which characterize developmental neuropsychiatric disorders, including autism, attention deficit disorder (ADD) , dyslexia and dyscalculia. Additional interests are adult outcomes in autism and dyslexia and relationships between patterns of neuropsychological deficits, psychiatric symptoms, and neurophysiological abnormalities. Ongoing studies of learning disabilities will attempt to determine both the sensitivity and diagnostic specificity of ERP and EEG spectral analysis and the role of attentional dysfunction in various subtypes of learning disorders.

Methods: Methods include EEG spectral analysis combined with topographic mapping, event-related potentials (ERPs), the measurement of regional cerebral blood flow using xenon inhalation (all of preceding are used in conjunction with activation tasks), quantitative analysis of MRI and CT scans, quantitative physical neurological examinations for soft signs, neuropsychological assessment, structured psychiatric interviews, standardized rating scales for evaluating thought disorders and affective flattening, and behavioral questionnaires.

Major Findings: The following numbers of subjects have been evaluated as part of our ongoing studies of developmental dyslexia in adult men:

Procedure	Dyslexic patients	Normal Controls
EEG spectral analysis	12	4
Evoked potentials (3 sessions)	8	3
Regional cerebral blood flow (xenon inhalation method)	10	2
Magnetic resonance head scans	12	0
Quantitative neurologicals	13	4
Neuropsychological testing	10	4
ADD evaluation (questionnaire and interview)	9	3

MRI head scans on 12 dyslexic men are now being reviewed by two radiologists. However, preliminary impressions suggest normal macroscopic brain anatomy, as studied with MRI inversion recovery and spin echo techniques, in severe developmental dyslexia. Only one patient showed a gross lesion, and this very likely represents an incidental finding.

A preliminary (qualitative) inspection of EEG spectral data on 12 dyslexics and four controls during verbal and spatial memory tasks, as well as during a baseline resting condition, suggests no gross EEG abnormalities in the majority of patients. A single patient showed epileptiform discharges (in the absence of clinical seizures), but this likely represents an incidental finding. Some unusual asymmetry in the resting state is noted in three dyslexic patients. EEG spectral measures thus far appear fairly symmetrical under the verbal and spatial memory activation conditions. However, quantitative EEG image analysis has not been completed yet, as additional control subjects are needed.

Performance measures taken in conjunction with the activation tasks show marked verbal memory deficits in only three patients. This suggests that our attempts to design a verbal task that dyslexics could perform (by limiting the reading level to a fourth grade level and under) have been fairly successful. A decrement in the performance of dyslexics is seen when nonsense syllables are used in place of low-level reading vocabulary.

ERP data suggest a reduction in the amplitude of P300, a sensitive index of attention, in dyslexia. Abnormalities become more pronounced with increasing attentional demands.

A preliminary examination of cerebral blood flow data on our first six dyslexic men shows somewhat higher blood flow in the left hemisphere, as compared with the right, on our "numbers" control task. This coincides with previous data collected on normal controls in other studies. This task requires minimal cognitive activity (the subject merely views letters and selects a lever numbered to match his choice). The sensory input and motor output involved here are the same as that required by our experimental tasks. Our "right hemisphere" task is an adaptation of Benton's line orientation (a task shown to be sensitive to acquired right hemisphere lesions). Here our dyslexic subjects show somewhat higher blood flows in their right, as compared with their left, hemispheres. Our "left hemisphere" task ("semantic classification") requires the subject to read a word and then to select the category to which it belongs. All words fall within a first to fourth grade reading level. Here, our dyslexic subjects showed somewhat higher flows on the left than on the right. In all three tasks, five out of six of these dyslexic subjects showed differences that fall in the above directions, thus indicating considerable consistency. The left-right differences appear to be small, as small in the case of the experimental tasks as they are in the control task. Nevertheless, they fall in the directions expected for normals. Because of the limited number of control subjects tested, we cannot yet tell whether these small left-right hemisphere differences in the dyslexics are abnormally low (that is, whether dyslexics show abnormally symmetrical flows). In addition, we need to examine values for specific regions within each hemisphere to determine whether any differences between our groups are more posterior than anterior, as hypothesized.

In autism, volumetric CT studies show relatively normal brain anatomy, with the exception of enlargement of the left lateral ventricle. Neuropsychological deficits in high-functioning autistic adults resemble those seen in frontal and subcortical disorders. Our psychiatric studies show that high-functioning autistic adults share certain negative symptoms with schizophrenics -namely, negative thought disorders and affective flattening, but positive thought disorders, seen in both schizophrenia and mania, are absent in autism.

Significance to Mental Health Research: Available anatomical techniques (CT and MRI) appear to hold limited diagnostic and localization value for these developmental disorders. Physiological techniques, particularly when used with neuropsychological activation procedures, may provide more sensitive tools for localizing dysfunction in these disorders. Autism and schizophrenia may share

a variety of deficit symptoms (e.g., negative thought disorders, affective flattening, and neuropsychological deficits remniscent of frontal-subcortical disorders) though dissociated by the presence of positive thought disorder. This provides validating evidence for current diagnostic distinctions found in DSM-III, but raises the possibility of some shared substrate.

Proposed Course of Project: We plan to complete our series of studies on developmental dyslexia in adults by early 1986 and then begin our studies of learning and attentional disorders in children. Future possibilities also include a study of brain metabolism in dyslexia using positron emission tomography (PET). The scheduled installation of a high-resolution PET-scanner in January, 1986, together with the recent installation of two cyclotrons, making the use of radioisotopes with short half-lives feasible, will provide an opportunity to perform three-dimensional examinations with good resolution in conjunction with neuropsychological activation procedures.

Publications:

Rapoport, J. L., Rumsey, J., Duara, R., Schwartz, M., Kessler, R., Cutler, N. & Rapoport, S. I.: Cerebral metabolic rate for glucose in adult autism as measured with positron emission tomography (PET). J. Cereb. Blood Flow and Metab. 3 (Suppl. 1): S264-S265, 1983.

Rumsey, J.: Conceptual problem-solving in highly verbal, nonretarded autistic men. J. of Autism Dev. Disord. 15 (1): 23-36, 1985.

Rumsey, J., Andreasen, N. & Rapoport, J. L.: Thought, language, communication, and affective flattening in autistic adults. Arch. Gen Psychiatry. In press.

Rumsey, J. & Denckla, M.B.: Neurobiological research priorities in autism. In Schopler, E. & Mesibov, G. (Eds.): Current Issues in Autism, in press.

Rumsey, J., Duara, R., Rapoport, J.L., Schwartz, M., Kessler, R., Cutler, N. & Rapoport, S. I.,: Brain metabolism in autism: Resting cerebral glucose utilization rates as measured with positron emission tomography (PET). Arch. Gen. Psychiatry. 42: 448-455, 1985.

Rumsey, J., Grimes, A., Pikus, A., Duara, R., Rapoport, J. L. & Ismond, D.: Auditory brainstem responses in pervasive developmental disorders. Biol. Psychiatry. 19: 1403-1418, 1984.

Rumsey, J. & Rapoport, J.: Assessing behavioral and cognitive effects of diet in pediatric populations. In Wurtman, R.J. & Wurtman, J.J. (Eds.): Nutrition and the Brain, Volume VI. New York, Raven Press, 1983, pp. 101-161.

Rumsey, J., Sceery, W. & Rapoport, J.L.: Autistic children as adults: Psychiatric, social, and behavioral outcomes. J. Amer. Acad. Child Psychiatry. 24: 465-473, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00301-03 CHP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnosis in Child Psychiatry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith L. Rapoport, M. D., CHP, NIMH
Maureen Donnelly, M. D., Clinical Associate, CHP, NIMH
Alan J. Zametkin, M. D., Staff Psychiatrist, CHP, NIMH
Mary Beth Ahearn, Ph.D., Psychologist, Johns Hopkins School of Mental Hygiene
Eric Taylor, M. D., Senior Registrar, The Maudsley Hospital, London
Michael Pendergast, M. D., Registrar, The Maudsley Hospital, London
Michael Rutter, M. D., Professor of Child Psychiatry, The Maudsley Hospital, London

COOPERATING UNITS *(if any)*

Johns Hopkins University, School of Mental Hygiene, Baltimore, Maryland; Department of Psychiatry, Maudsley Hospital, London

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.40	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A case history exchange was carried out to study the diagnosis of hyperkinesis (Attention Deficit Disorder) and Conduct Disorder in Europe and the United States. Both research teams at the NIMH and the Maudsley Hospital in London, as well as clinician teams in Washington, D. C. and London, rated 40 case histories, 20 with videotapes. Both the International Classification of Disease - 9th edition (ICD-9) and Diagnostic and Statistical Manual (DSM III) were used by all raters.

Preliminary data analysis shows that much of the wide discrepancy between European and U. S. rates is due to the different schemes involved. ICD-9 only permits one diagnosis and Conduct Disorder is so defined as to be quite inclusive. Thus, the research teams agreed well within schemes, both diagnosis Conduct Disorder more frequently using DSM III which permits multiple diagnoses. Agreement was good between research teams within each scheme.

Preliminary analyses of the clinician team data shows both influence of diagnostic schemes as well as an influence of the nationality of the clinician. While still diagnosing Attention Deficit Disorder more frequently using DSM III, British clinicians also implement the schemes differently.

Project Description:

Objectives: To examine the widely discrepant rates of diagnosis of childhood hyperactivity between the U.S. and most European countries; the U.S. rates are more than 15 times greater than that reported for Europe. Differences in the schemes (DSM III for the U.S. and ICD-9 for Europe) as well as implementation of the schemes were explored.

Method Employed: A total of 40 case histories were prepared, 20 from the Child Psychiatry program at the NIMH and 20 from the Maudsley Hospital, Division of Child and Adolescent Psychiatry. A brief (three page) case history was prepared for all cases according to a standardized format; videotaped interview using the Rutter semi-structured interview format, was prepared for 10 cases from each center.

The cases were rated both by research teams at the Maudsley and at the NIMH following preliminary training at both sites. Subsequently, two large clinician teams of senior specialists in child psychiatry also rated all cases; 23 clinicians in the U.S. and 21 clinicians in London completed the ratings.

Analysis of the research teams diagnoses has been completed. There was surprisingly good agreement of research teams within schemes. That is, on Axis I of DSM III the teams had a kapa of .69 (for 3 digit agreement). For Axis I of ICD 9 the equivalent figure is .60 and even this corresponds to perfect agreement on better than 80% of the cases. The nature of the disagreement seems to be with mixed cases - deciding where to place a mixed case. Within ICD 9, 22 cases were rated as having hyperactivity while 33 were rated within DSM III. The discrepancy is not large compared to the discrepancy seen across countries.

Preliminary analysis of clinicians' ratings indicates considerable clinician bias as well as that attributable to schemes, particularly for ICD 9.

Major Findings: The major source of discrepancy between the U. S. and UK seems to be provided by the different diagnostic schemes. However, differences between clinicians, and possibly in referral pattern are also found.

Significance to Mental Health Research: Childhood hyperkinesis has been considered an American phenomenon and widely ascribed to dietary, cultural and/or environmental factors. The present study suggests that the children do not differ so widely as had been supposed and that most of the difference is accounted for by clinician rating scheme and possibly referral differences in the two countries. Epidemiological data must first refine the definition so that it is acceptable to all participants before any World Health Organization sponosored study could commence.

Proposed Course of Project: Following analysis of the clinician teams, children will be selected on both sides of the Atlantic and more direct observations compared, including actometer rated motor activity. In addition, the Child Psychiatry Branch at NIMH is participating in field trials within the U.S. of revised DSM III criteria for Attention Deficit Disorder that may be more in accord with European schemes.

Publications:

Taylor, E. & Rapoport, J.L.: Diagnosis of hyperactivity - U.S.-UK differences. In J. Sargeant and L. Bloomingdale (Eds.): Research Diagnostic Criteria for Attention Deficit Disorder. New York, Spectrum Publications, in press.

Rapoport, J.L.: DSM III-R and child diagnosis. In C. Last and M. Hersen (Eds.): Issues in Diagnostic Research. New York, Academic Press, in press.

Rapoport, J.L.: DSM-III-R and pediatric psychopharmacology. Psychopharm. Bull., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02240-01 CHP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurobiology of Attention Deficit Disorder

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith L. Rapoport, M.D., CHP, NIMH
Alan J. Zametkin, M.D., Staff Psychiatrist, CHP, NIMH
Markus J. P. Kruesi, M.D., CHP, NIMH
William Z. Potter, M.D., Ph.D., Chief, Section on Clinical Pharmacology, LCS, NIMH
Markku Linnoila, M.D., Ph.D., Chief, LCS, NIAAA
Robert M. Cohen, M.D., Ph.D., Chief, Section on Clinical Brain Imaging, LCM, NIMH

COOPERATING UNITS *(if any)*

Section on Clinical Pharmacology, LCS, NIMH
National Institute on Alcohol Abuse and Alcoholism
Section on Clinical Brain Imaging, LCM, NIMH

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	.75	.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Attention Deficit Disorder with Hyperactivity (ADDH) is a life-long disability affecting approximately 4% of school age children. To understand the pathophysiology of this disorder and to develop new treatments, a therapeutic trial of the amino acid, d-phenylalanine, was completed over the past nine months. Eleven ADDH boys participated in a placebo controlled double blind evaluation of phenylalanine (10/mg/kg/day) and placebo. Behavioral data and biochemical measures in urine and plasma are being analyzed at present and results are forthcoming.

In an attempt to localize abnormal patterns of cerebral glucose metabolism and cerebral flow in adults with Attention Deficit Disorder (ADD) Residual Type, fathers of diagnosed hyperactiave children are being studied using the 2-deoxyfluoroglucose method of Positron Emission Tomography (PET). To date, four patients have been scanned.

Because of reports that the spinal fluid (CSF) 5HIAA, a serotonin metabolite, may be low in severely impulsive or aggressive adults, a study has been initiated to compare spinal fluid catecholamine and indoleamine metabolites in severely aggressive, conduct disordered children with and without ADD to children with other neurological problems. Peripheral measures of catecholamines will also be obtained.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Objectives: The major objectives are to delineate the pathophysiology of ADDH. Specific biochemical hypotheses are tested using a variety of pharmacological and other interventions such as amino acid precursor treatment. Secondary objectives include development of more effective treatments for this disorder that might lack specific side effects of standard treatment.

Methods Employed: Three approaches are being used. First, a specific agent with well documented biochemical effects in a therapeutic trial. The amino acid, phenylalanine (20 mg/kg/day), which is a precursor to catecholamines, was given to 11 hyperactive children between the ages of six and 13 with carefully diagnosed Attention Deficit Disorder, in a double blind, placebo controlled crossover trial in which parent and teacher behavior rating, attention memory and learning, were measured as well as plasma and urine amino acids, catecholamines and trace amines.

Second, spinal fluid and plasma catecholamines and indoleamines between children with severe aggression and/or Attention Deficit Disorder and neurological controls are being compared. Other centers have reported plasma and urinary differences between ADDH children and controls and the adult literature has found spinal fluid differences in aggressive adults. In this study, children will be admitted to the Clinical Center for a medical, psychiatric, and psychological and assessment, as well as for blood sampling and lumbar puncture exam for catecholamines and indoleamines.

Major Findings: Eleven children have completed the phenylalanine trial to date. Data analysis is not complete, and biochemical analysis of blood and urine parameters will be starting shortly. The clinical impression however, is that phenylalanine is ineffective. There were no major side effects and all children tolerated the amino acid well. No marked deterioration was seen.

Results of the Positron Emission Tomography (PET) are not yet available, and only four scans have been completed. However, the clinical assessment of these parents of hyperactive children have confirmed earlier studies demonstrating the life-long disabilities these individuals may have.

The study of peripheral and spinal fluid measures of Attention Deficit Disorder with and without severe aggression will begin in August, 1985.

Significance to Mental Health Research: Attention Deficit Disorder is a relatively common childhood disability that persists into adulthood for about 30% of the cases. The phenylalanine trial in the treatment of childhood ADDH provides the possibility of alternative treatment if proven efficacious, and clarifies biochemical hypothesis regardless of treatment outcome. From a public health perspective, phenylalanine is of interest in that it is commonly ingested as a food additive called Aspartame.

To date, there had been only one study that attempt to localize an area of cerebral dysfunction in patients with ADDH. If this study replicates an earlier study (in ADDH children) of decreased cerebral blood flow in frontal area, research can focus more intensely on frontal lobe function.

Proposed Course of Project: The phenylalanine trial has just been completed. Baseline clinical measures, plasma phenylalanine levels, and urinary amines will be correlated with significant behavioral changes, if any, on phenylalanine.

The PET scan project will examine glucose metabolism in 10 adults with ADDH Residual Type. Cerebral blood flow and glucose uptake will be compared with that of age matched normal controls. Personality parameters and scores on a measure of attention, the Continuous Performance Test, will be examined in relationship to the scans.

The study of peripheral and spinal fluid measures of catecholamines and indoleamines has just begun. It is expected to take approximately three years to complete and will require 75 patients with ADDH and/or severe conduct disorder, and 75 patients with other neurological problems as controls.

Publications:

Rapoport, J. & Zametkin, A.: The psychopharmacological investigation of attention deficit disorder. In Bloomingdale, L. M. (Ed.): Attention Deficit Disorder, New York, SP Medical and Scientific Books, in press.

Zametkin, A. & Rapoport, J.: The pathophysiology of attention deficit disorder with hyperactivity: A review. In Lahey, B. and Kazin, A. (Eds.): Advances in Clinical Child Psychology, Vol. 9, New York, Plenum Publishing Corp., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00084-11 CNG

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic-Biologic Studies of Psychiatric Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	E. Gershon	Chief	CNG, NIMH
Others:	L. DeLisi	Staff Psychiatrist	CNG, NIMH
	J. I. Nurnberger, Jr.	Medical Officer	CNG, NIMH
	W. H. Berrettini	Staff Psychiatrist	CNG, NIMH
	J. Hamovit	Research Social Worker	CNG, NIMH
	L. Goldin	Senior Staff Fellow	CNG, NIMH
	J. Baumgold	Research Chemist	CNG, NIMH

COOPERATING UNITS *(if any)*
LCS, NSB, NIMH; LCS, NIAAA; Yale University; Washington University; Jerusalem Mental Health Center; INSERM

LAB/BRANCH
Clinical Neurogenetics Branch

SECTION
Section on Clinical Genetics

INSTITUTE AND LOCATION
NIMH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.8	4.5	4.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Controlled family studies of schizophrenia and of bulimia are underway. In the schizophrenia study 51 patients and 28 normals have been interviewed, as well as 136 of their relatives; the bulimia study is in the planning stage. Family study of affective disorders suggests that a restrictive definition of major depression, which requires impairment or incapacitation in the person's major social role, may usefully identify cases with a familial, presumably genetic vulnerability.

Two new polymorphisms have been identified on two-dimensional electrophoretic gels bringing the total number identified to 27, and two new linkages of new protein polymorphisms to known markers were identified. Other analytic methods assign a mixed single-locus/polygenic inheritance mode to catechol-O-methyltransferase and to dopamine beta-hydroxylase. Previously, only the single locus component to their inheritances was known.

Receptor-like characteristics identified on adult skin fibroblasts include specific and saturable binding of vasoactive intestinal peptide (VIP), as well as VIP stimulated release of arachadonic acid. Somatostatin (SRIF) shows specific binding, as well as inhibition of cyclic guanine monophosphate (cGMP) production in these cells. Muscarinic receptor-like characteristics, at this point, are confined to antagonist binding.

In other studies, heritability of forskolin-stimulated but not hormone stimulated adenylate cyclase in lymphocytes was demonstrated.

Other Investigators (Continued):

C. Merril	Chief, Section on Biochemical Genetics	CNG, NIMH
J. Bardakjian	Visiting Fellow	CNG, NIMH
D. C. Jimerson	Chief, Section on Experimental Therapeutics	LCS, NIMH
D. Pauls	Assistant Professor	Yale Univ.
K. Kidd	Professor	Yale Univ.
N. Cox	Post-Doctoral Fellow	Washington Univ.
D. Goldman	Chief, Unit on Genetic Studies	LCS, NIAAA
D. Pickar	Chief, Sect. on Clinical Studies	NSB, NIMH
R. Ebstein	Director, Geriatric Research, Jerusalem Mental Health Ctr.	
A. Price	Post-doctoral Fellow	Yale Univ.
F. Clerget-Darpoux	Geneticist	INSERM, France
D. Pickar	Chief, Section on Clinical Studies	NSB, NIMH
J. Schreiber	Research Social Worker	NSB, NIMH

1. Family Studies

A. Schizophrenia

Family diagnostic studies are being performed on patients admitted to the Clinical Center (in collaboration with Dr. David Pickar) and patients admitted to Chestnut Lodge and Springfield State Hospital.

Families of psychiatrically normal individuals are studied as a control group. The study includes use of a separate diagnostic instrument for schizotypal and other personality disorders, to determine if there is familial aggregation of these disorders with schizophrenia.

At this point, chart review has identified 139 patients suitable for study, and 28 controls. Fifty-one patients have been enrolled in the study, and 82 relatives of these patients have been examined, as well as 28 controls and 54 of their relatives.

Other studies of multiplex families (multiple ill members) and extended pedigrees are described in project report Z01 MH 02236-01 CNG (Dr. DeLisi).

B. Bulimia

In collaboration with Dr. David Jimerson, a family study of mood and eating disorders, in relatives of normal weight bulimics, has begun.

C. Affective Disorders

The DSM-III criteria for major depression may be criticized for including much milder episodes than were ever accepted in classic family studies of affective illness. In our family studies, we have required impairment or incapacitation in the principal social role of an individual,

as an additional criterion to the DSM-III criteria for major depression; we also require 4 weeks duration.

As compared with depressed relatives of normals, depressed relatives of affective patients are more likely to have severe impairment or incapacitation when depressed, and more likely to suffer multiple episodes. These findings suggest that among major depressions, these clinical criteria may usefully identify cases with a familial, possibly genetic, vulnerability.

D. Other Studies

A cell collection of extended pedigrees of manic-depressive patients and schizophrenics is being prepared in collaboration with NIGMS. These cells have been used to demonstrate new DNA polymorphisms and to test for linkage of neuropeptide and other genes to psychiatric illness (see report Z01 MH 02237-01 CNG)

Also, see Z01 MH 00086-09 CNG, High Risk Study of Affective Disorder.

2. Population Genetic Studies (Drs. Goldin and Gershon)

A. Analysis of Linkage Relationships among Newly Identified Protein Polymorphisms

New protein polymorphisms had previously been detected by two-dimensional electrophoresis of lymphocytes, fibroblasts, serum, and erythrocytes (see Z01 MH 02238-01 CNG).

Two new polymorphisms have been identified from two-dimensional electrophoresis of fibroblasts and serum since the last report. This brings the total number of polymorphisms identified to 27. We have studied linkage relationships of these polymorphisms to 19 classical marker loci in a second pedigree of approximately 40 individuals. We confirmed the identity of glyoxalase-1 in red cells and phosphoglucomutase-3 in fibroblasts. We found a possible linkage of an unknown serum locus (SER 1) to alpha-haptoglobin (maximum lod score = 1.9 at $\theta m = .1$, θ_f 0). An unknown fibroblast protein (NC22) may be linked to apolipoprokein E detected in serum (maximum lod score = 2.8 at $\theta m = \theta_f = 0$). We have also been able to determine the identities of two previously unidentified serum loci, apolipoprotein E and apolipoprotein A4. Six of 17 polymorphisms detected in fibroblasts were positionally identical to polymorphic loci seen in lymphocytes. These studies indicate a minimum level of average protein charge heterozygosity of approximately 2.2% for the most predominant human cellular proteins and of 5.6% for the most predominant proteins of serum. These results are currently in press in the American Journal of Human Genetics.

B. Segregation Analysis of Catecholamine Enzymes

Activity of enzymes of catecholamine metabolism, plasma dopamine-beta-hydroxylase (DBH) and erythrocyte catechol-O-methyltransferase (COMT) were each

previously shown to be transmitted as single codominant loci in a sample of approximately 30 multigenerational families that were analyzed with the single major locus model.

We have recently re-analyzed these data using the mixed model of transmission which has the advantage of allowing one to test the significance of both major locus and polygenic transmission. The computer program POINTER was used to estimate parameters and calculate likelihoods for each hypothesis. For plasma DBH, the most parsimonious model was a dominant major locus (i.e. high values dominant to low values) accounting for 41% of the variance and a polygenic component accounting for 25% of the variance. For erythrocyte COMT, the most parsimonious model was a dominant major locus accounting for 56% of the variance and a polygenic component accounting for 27% of the variance. The major locus for COMT has been supported by previous biochemical studies. The major locus for DBH is supported by the finding from our previous study of possible linkage to the ABO locus. This analysis shows the power of the mixed model procedure to detect both major locus and polygenic transmission.

C. Heritability of Forskolin and Hormone-stimulated Adenylate Cyclase Activity in Human Lymphocytes

Isoproterenol, prostaglandin E_1 and forskolin-stimulated cyclic AMP accumulation were compared in intact lymphocytes obtained from nine monozygotic and nine sib pairs matched for age and sex. Heritability was calculated by three different methods, two based on the intraclass correlation coefficients and one based directly on the variances. Only for forskolin is a significant percentage of variance (0.68-0.91) attributable to genetic factors, suggesting that forskolin-stimulated activity may prove to be a valuable genetic marker in studies of human pathology. Neither basal nor isoproterenol and prostaglandin E_1-stimulated activity show significant heritability in intact human lymphocytes. The individual differences observed in levels of beta-adrenergic and prostaglandin stimulated receptor activity in human lymphocytes are, therefore, most likely due to environmental factors.

D. Multiple Threshold Models for the Affective Disorders

From the Yale-NIMH collaborative family study, 1482 first degree relatives of 90 bipolar I, and 163 major depression probands were examined to test the hypothesis that bipolar I and major depression are due to a single underlying genetic liability. We attempted to fit multifactorial-polygenic and single-major-locus multiple threshold models for sex and severity to the relatives in the major depression and bipolar I families. With relatives classifed as affected only if they met criteria for major depression or bipolar I, there was at best only marginal support for these models. Differences between these and previously reported results were examined in relation to differences in underlying assumptions. Additional analyses of these and other data from families of NIMH bipolar II and schizoaffective probands suggest that different methods of age

adjustment, the relative placement of bipolar II and schizoaffective disorders in a hypothesized liability continuum and the inclusion or exclusion of sex thresholds were not primarily responsible for differences in the fit of genetic threshold models. Factors which do appear to be important include the "cohort effect" that is, the presence of a large secular increase in affective illness over the past three generations; the secular trend does not conform to the models of genetic transmission examined.

E. Critique of Clinical Methods in Psychiatric Genetics

A series of three papers has been submitted for publication on the power of currently used investigative strategies. For heritable risk factors, that are not determined by single genetic loci, the strategy of examining ill and well relatives for the risk factor is most robust, except when the putative risk factor is a secondary effect of illness or treatment. Examination and follow-up of young persons at risk avoids this problem. The least robust strategy is division of patients into family history present and absent, since either group can have genetic illness.

3. Receptors and Receptor-Related Events in Adult Skin Fibroblasts (Dr. Berrettini)

A. Vasoactive Intestinal Peptide (VIP)

We have demonstrated specific, reversible, and saturable binding of VIP to fibroblasts (k_D = 22.3 pm, B_{max} = 27.6 fmoles/mg protein). Subsequently we determined that VIP increases, in a dose-dependent manner, the release of arachidonic acid from the fibroblast cell, probably through an activation of a membrane-bound phospholipase A_2 (PLA_2). Studies on the pharmacology and stability of these observations are currently being conducted.

B. Somatostatin (SRIF)

Specific reversible binding of SRIF to fibroblasts has been demonstrated although saturability has not been clearly evident. In addition SRIF has been shown to inhibit cGMP production in these cells. Studies of the pharmacology and stability of these observations are currently being conducted.

C. Muscarinic Receptors

Unfortunately, we are no longer able to demonstrate functional muscarinic receptors on the adult skin fibroblasts. Specific and saturable binding of antagonists, QNB and N-methylscopolamine, can be demonstrated when 1mM or higher concentrations of atropine are used to define non-specific binding. The Scatchard analysis of these experiments was consistent with two classes of binding sites, one high affinity (40 pM QNB), low capacity (100 fmoles/mg protein) site and one low affinity, essentially unsaturable

site. The high affinity site disappeared if the cells were grown to confluence in the wells in which the assay was performed. Two sites were consistenly found if the assay was performed prior to confluence.

Attempts to up or down regulate these sites by incubating the fibroblasts with cholinergic antagonists (atropine) or agonists (arecoline) were not successful.

The binding of various cholinergic agonists to fibroblasts has also been studied. No specific binding could be demonstrated for two agonists, McN-A-343 and oxytremorine. Propylbenzylcholine mustard, a covalently binding muscarinic ligand, did not bind to the fibroblast cell membranes, Cholinergic agonists did not produce any change in baseline cGMP production in these cells nor did they alter PLA_2 activity. Studies of cholinergic influence on cAMP production are currently being conducted.

Activity of membrane-bound PLA_2 is thought to be functionally related to the number of muscarinic receptors on cultured guinea pig muscle cells. Given the observation that cells from manic-depressive patients may have increased numbers of QNB binding sites on fibroblasts compared to normal volunteers, we determined fibroblast membrane-bound PLA_2 activity in cells from three large pedigrees of manic-depressive probands. No differences were found between patients and controls.

It is not clear why our current results are so different from those reported a year ago, when single-site saturable binding of antagonist ligands was demonstrable at much lower concentrations of displacing agent, and physiologic evidence of receptor function was demonstrated. We are investigating different conditions of cell incubation, and continuing our studies of muscarinic receptor-like characteristics in these cells, to resolve these inconsistencies.

4. Purification of Muscarinic Receptor Protein (Dr. Baumgold)

Two separate approaches have been pursued in the purification of the muscarinic receptor protein from rat and from bovine brain. Our goal in this work has been to purify sufficient receptor protein to enable us to get it microsequenced. In one approach, the receptor was affinity labeled with ^{3}H-propylylbenzylcholine mustard, a specific alkylating agent for this protein. The labeled receptor was partially purified using wheat-germ lectin chromatography and preparative gel electrophoresis. Attempts at using preparative isoelectric focusing to purify this labeled receptor were, until recently, unsuccessful. In the other approach, the free receptor was solubilized and purified using ion-exchange, hydroxylapatite, and affinity chromatography. The receptor may be purified approximately 500 fold using these techniques, and was then purified to homogeneity using gel electrophoresis. The yields in this approach have been quite low, but work is currently underway to improve these yields.

5. Molecular Genetic Studies (see Z01 MH 02237-01 CNG).

Significance to Biomedical Research and the Program of the Institute:

Successful identification of a marker of genetic vulnerability to psychiatric disorders would lead to identification of the responsible pathophysiological process, and would have clinical applications for prevention and for choice of treatment. Identification of new polymorphisms and establishment of linkage relationships is a necessary scientific underpinning for this goal. Development of cellular models of CNS receptors has importance for clinical and genetic investigation of these receptors in disease. Family diagnostic studies are necessary to provide a spectrum of clinical conditions to test for association with altered pathophysiological characteristics or linkage markers.

Proposed Course of Project:

We plan to continue to investigate the biology and genetics of characteristics that may be implicated in the genetics of affective disorders, as described above. Further study of the fibroblast as a clinical neuronal model will proceed. The molecular genetics approach of interindividual differences in neuropeptides and other substance will be pursued. Establishing a library of DNA and living cells from entire pedigrees is a major priority. Study of relatives at risk for affective disorders and schizophrenia will proceed. Mathematical methodology for clinical investigation will continue to be studied.

Several projects have been spun off from this one: Z01 MH 02236-01 CNG, Schizophrenia Studies, Z01 MH 02237-01 CNG, Molecular Genetics of Neuropsychiatric Disorders and Z01 MH 00086-09 CNG, Outpatient Clinic for Genetic and Pharmacological Studies of Affective Disorders, High Risk Study of Affective Disorder. This project will be continued as the main biologic-genetic project of the Section, and the start-up of new initiatives will be within this project.

Publications:

Goldin, L.R., Cox, N.J., Pauls, D.L., Gershon, E.S., and Kidd, K.K. The detection of major loci by segregation and linkage analysis: A simulation study. Genetic Epid. 1: 285-296, 1984.

Del Zompo, M., Bocchetta, A., Goldin, L.R., Corsini, G.U. Linkage between X-chromosome markers and manic depressive illness. Two Sardinian pedigrees. Acta Psychiatr. Scand. 70: 282-287, 1984.

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Goldin, L.R. Segregation analysis of dopamine-beta-hydroxylase (DBH) and catechol-O-methyltransferase (COMT): Identification of major locus and polygenic components. Genetic Epidemiology, in press.

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Nadi, N.S., Nurnberger, J.I., Jr., Gershon, E.S.: Muscarinic cholinergic receptors on skin fibroblasts in familial affective disorder. N. Engl. J. Med, 311: 225-230, 1980. Also see Letters to Editor, N. Engl. J. Med. 312: 861-862, 1985.

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Stessman, J., Mintzer, J., Lipschitz, Y., Shemesh, Z., Goldin, L.R., Ebstein, R.P.: Heritability of forskolin and hormone stimulated adenylate cyclase activity in human lymphocytes. J. Cyclic Nucleotide and Protein Phosphorylation Research, in press.

Gershon, E.S., Weissman, M.M., Guroff, J.J., Prusoff, B.A., Leckman, J.F.: Validation of criteria for major depression through controlled family study. J. Aff. Dis., in press.

Gershon, E.S.: Genetic perspectives. In Goodwin, F.K., and Jamison, K.R. (Eds.): Manic-Depressive Illness. London, Oxford University Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00085-09 CNG

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacogenetics of Psychoactive Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J.I. Nurnberger, Jr.	Medical Officer	CNG, NIMH
Others:	S. Simmons-Alling	Clinical Nurse Expert	CC, NIH
	W. Berrettini	Staff Psychiatrist	CNG, NIMH
	E. Gershon	Chief	CNG, NIMH

COOPERATING UNITS *(if any)*

CC, NIH; CPB, NIMH; LN, NIAAA; University of Oregon

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.1	.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two of the most consistent biologic abnormalities found during depression are excess production of cortisol and decreased latency to rapid-eye movement (REM) sleep. We are employing a pharmacologic challenge strategy to attempt to elicit similar abnormalities in well state patients off medications.

The muscarinic cholinergic agonist arecoline is being used to provoke REM in sleeping subjects. Thirty patients and 21 controls have participated in this ongoing project.

The serotonin precursor tryptophan and the opiate antagonist naloxone have been used to probe cortisol secretion. Dose-response studies have been completed. Both agents provoke significant increases in cortisol. Patient versus control comparisons are in progress.

Beta endorphin levels have been reported elevated in affective patients, both at base-line and in response to cholinergic stimulation. We have demonstrated beta endorphin elevations in response to naloxone and to high doses of arecoline and tryptophan. In the latter two paradigms beta endorphin elevation appears related to a stress response.

The serotonin receptor blocker metergoline appears to block the dextro-amphetamine-induced rise in cortisol in preliminary data analysis. This observation, if substantiated in further testing, would conclude our neurochemical analysis of amphetamine effects.

OTHER INVESTIGATORS (CONTINUED)

A. Lewy	Associate Professor	University of Oregon
D. Sack	Chief, Inpatient Unit	CPB NIMH
W. Mendelson	Chief, Unit on Sleep Studies	CPB NIMH
T. Soncrant	Staff Fellow,	LN NIAAA

Project Description

1. Pharmacologic dissection of amphetamine response

We have previously demonstrated that the behavioral excitation response to amphetamine is blocked by the dopaminergic antagonist haloperidol, and the blood pressure and norepinephrine responses by the beta-adrenergic receptor antagonist propranolol. Hormonal responses were not blocked by either of these agents, nor were they blocked by the alpha-adrenergic antagonist thymoxamine.

There is evidence that amphetamine releases serotonin as well as dopamine and norepinephrine, and serotonin may in turn cause the release of pituitary hormones. A study is in progress to determine whether the serotonin receptor blocker metergoline will prevent the rises in cortisol, prolactin, and growth hormone that are caused by amphetamine. Preliminary data analysis suggests that metergoline blocks cortisol response. Ten persons have participated in 34 infusions related to this study. Further analysis of hormonal results is planned.

2. ACTH stimulation

It is known that cortisol is excessively secreted by depressed patients. This appears to be caused by excess release of ACTH from the pituitary gland. We are examining various neurochemical agents that are known to provoke ACTH release in order to see whether euthymic (well-state) bipolar patients are more sensitive to these agents. An abnormal response in well-state patients might be a clue to an underlying genetic vulnerability factor for affective disorder.

The opiate antagonist naloxone and the serotonin precursor tryptophan have been reported to cause release of ACTH. We have performed 31 infusions on 13 subjects with naloxone and 37 infusions on 15 subjects with tryptophan in an effort to find a dose near the threshold for ACTH stimulation. Tryptophan causes a dose-related increase in cortisol but not beta-endorphin. Naloxone at 0.125 and 0.25 mg/kg causes a significant increase in cortisol. The larger dose also causes an increase in beta endorphin. Further hormonal analyses of these samples, and examination of the data for patient-control differences, is planned.

3. Cholinergic REM induction

We have previously reported that well state bipolar patients were more sensitive than normal volunteers to the REM (rapid eye movement sleep)

inducing effects of the muscarinic cholinergic agonist arecoline. In this study, a single dose of 0.5 mg arecoline is given intravenously to a sleeping subject 25 minutes after the end of the first REM period. The latency to the beginning of the second REM period is measured. Thus far, thirty patients and 21 normal volunteers have participated. When the quality of sleep was adequate for the drug to be given and the response scored, seven of 12 patients were quick REM inducers or awakened in response to the drug. Of the controls, 5 of 15 were quick REM inducers or awakened. Data from other studies suggests that awakening is analogous to REM induction. We are continuing this work and plan analysis of baseline sleep variables in these groups also. We have also shown that arecoline increases beta endorphin levels when given in higher doses.

Arecoline effects in rat brain have been examined using the quantitative (^{14}C) 2-deoxy-D glucose method 3 minutes after intraperitoneal administration of 0.05 to 50 mg/kg arecoline. Rats were pretreated with methylatropine 4 mg/kg subcutaneously. Nine areas thought to be involved in REM generation were examined. After 0.05 mg/kg of arecoline, increases ($p < 0.05$) in cerebral glucose utilization were seen in the dorsal and median raphe nuclei and in the pons. After 0.5 mg/kg, increases were also seen in the mesencephalic and anterior pontine reticular formation and in the dorsal tegmental nucleus. Only after higher doses were significant effects seen in the locus coeruleus, the posterior pontine reticular formation and the lateral pontine tegmentum. Doses of 5 mg/kg and above cause an activation in most brainstem regions.

4. Light suppression of melatonin

We have previously reported that bipolar patients are more sensitive than controls to the melatonin inhibiting effects of light. In rats it has been reported that the light inhibition effect may be mediated by acetylcholine (probably via nicotinic receptors). We are testing physostigmine to see if it mimics light in reducing melatonin in man. Four subjects have received 0.25 mg of physostigmine IV over 5 minutes (without peripheral blockade) with no distress. Thus far, however, we have not been able to demonstrate a physostigmine effect on melatonin. In a related study we are planning to restart the studies directly examining light effects on melatonin. We plan to study offspring of bipolar patients and controls, and may also study normal twins.

Significance to Biomedical Research and the Program of the Institute

The cholinergic REM induction studies suggest that muscarinic cholinergic supersensitivity may be responsible for genetic vulnerability to affective disorder. If this is the case, it would provide a stimulus for localizing the molecular events that predispose to depression. It also may provide clinically useful genetic vulnerability markers. This is the possibility we are pursuing in our study of high risk offspring. The physostigmine results may tie in with this same vulnerability factor. The rat brain studies provide a basic laboratory model in which central cholinergic effects may be studied.

The amphetamine studies provide data on neurochemical control of what may be heritable responses to this psychoactive drug in man.

Our studies of ACTH response may provide clues to the basis of abnormal ACTH and cortisol secretion in depressed patients.

Proposed Course of Project

The course of the cholinergic studies is outlined above. We are ready to begin two new studies: 1) an investigation of the calcium channel blocker diltiazem; 2) an ACTH infusion study, coupled with a multi-day steroid challenge.

Publications:

Nurnberger, J.I., Jr., Simmons-Allings., S., Kessler, L., Jimerson, S., Schreiber, J., Hollander, E., Tamminga, C.A., Nadi, N.S., Goldstein, D.S. and Gershon, E.S.: Separate mechanisms for behavioral, cardiovascular, and hormonal responses to dextroamphetamine in man. Psychopharmacology 84: 200-204, 1985.

Gershon, E.S. and Nurnberger, J.I., Jr.: Is there a cholinergic depression-stress system? Integrative Psychiatry 3(1):13-14, 1985.

Lewy, A.J., Nurnberger, J.I., Jr., Wehr, T., Pack, D., Becker, L.E., Powell, R.L., Newsome, D.: Supersensitivity to light may be a trait marker for manic-depressive illness. Am. J. Psychiatry 142(6):725-727, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00086-09 CNG

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Outpatient Clinic for Genetic and Pharmacological Studies of Affective Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J.I. Nurnberger, Jr.	Medical Officer	CNG, NIMH
Others:	L. DeLisi	Staff Psychiatrist	CNG, NIMH
	W. Berrettini	Staff Psychiatrist	CNG, NIMH
	E.S. Gershon, M.D.	Chief	CNG, NIMH
	S. Simmons-Alling	Clinical Nurse Expert	CC, NIH
	J.R. Hamovit	Research Social Worker	CNG, NIMH
	E. Maxwell	Social Worker	CNG, NIMH

COOPERATING UNITS *(if any)*

CC, NIH; CHP, BPB, LPP, LCS, NPB, LCS, CPB, NIMH; Catholic University; University of Caen, France.

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	1.5	2.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In our continuing clinical CSF research, we have completed studies of monoamines and their metabolites, GABA, and 13 peptides, in 35 normal volunteers and 25 lithium-treated euthymic bipolars (15 of whom also provided samples in the unmedicated state). Strong correlations were found among seven neuropeptides studied in these subjects: CRF, VIP, N-POMC (N-Terminal fragment of pro-opiomelanocortin), ACTH, beta-endorphin, beta-lipotropin and somatostatin. CSF levels of neuropeptide Y and alpha-MSH were determined in anorexic and bulimic patients and normal volunteers. No group differences were found.

Although ventricular enlargement has been reported in some affective patients, examination of computerized tomographic scans in our clinic patients has not shown evidence of increased ventricular size in comparison to controls.

A family study of rapid cycling bipolar illness is in progress among the clinic patients. A review of our clinic and family study records has thus far identified 28 bipolar patients as rapid cyclers in comparison with 130 who are not.

Phenylethylamine has been proposed to be an endogenous psychotomimetic agent. We have measured this substance in 24 hour urine samples and have not found a difference between patients and controls.

Our longitudinal biologic and psychosocial follow up study of offspring of bipolar parents continues. The prospective design should enable us to identify variables which are predictive of illness. Eighty-seven subjects are participating in this study at present.

Investigators

L. Goldin	Senior Staff Fellow	CNG, NIMH
M. Harrington	International Fogarty Fellow	CNG, NIMH
C. Merril	Medical Officer	CNG, NIMH
L. Sapin	Guest Researcher	CNG, NIMH
E. Hibbs	Psychologist	CHP, NIMH
R. Post	Chief	BPB, NIMH
W. Kaye	Staff Psychiatrist	LPP, NIMH
H. Gwirtsman	Medical Staff Fellow	LCS, NIMH
D. Pellegrini	Assistant Professor	Catholic University
L. Pons	Professor	University of Caen, France
T. Zahn	Research Scientist	LPP, NIMH
F. Karoum	Research Scientist	NPB, NIMH
D. Murphy	Chief	LCS, NIMH
R. Wyatt	Chief	NPB, NIMH
R. Golden	Clinical Associate	CPB, NIMH

Project Description:

We maintain an ongoing treatment clinic for 120 manic-depressive outpatients for the purpose of: 1) identifying potential markers of genetic vulnerability to affective disorder; and 2) studying the course and treatment of affective illness, especially bipolar disorder.

For purposes of comparison, we have 52 unrelated normal volunteers and 23 pairs of normal monozygotic twin volunteers.

1. Markers of genetic vulnerability to affective illness

We study primarily "well state" patients to determine those abnormalities that are most likely abiding characteristics of the illness.

A. CSF Studies (Dr. Berrettini)

In our continuing clinical CSF research, we have completed studies of monoamines and their metabolites, GABA and 13 peptides in 35 normal volunteers and 25 lithium-treated euthymic bipolars (15 of whom also provided samples in the unmedicated state). No group differences were seen across any measure. No effect of lithium was seen.

A series of remarkably strong correlations were found among seven neuropeptides studies in these subjects: CRF, VIP, N-POMC (N-Terminal fragment of pro-opiomelanocortin), ACTH, beta-endorphin, beta-lipotropin and somatostatin. Factor analysis revealed that one-half of the variance of each of these seven neuropeptides could be attributed to a single factor. Levels of other CSF neuropeptides (neurotensin, substance P, alpha-MSH, calmodulin, calcitonin, vasopressin, neuropeptide Y) were not related. This observation is consistent with the hypothesis that their release into CSF may be a functionally significant CNS process, as opposed to random diffusion from synaptic sites of release.

Eating disorders appear to be genetically related to affective disorders in our family data. CSF levels of neuropeptide Y and alpha-MSH were determined in anorexic and bulimic patients and normal volunteers in collaboration with Water Kaye, M.D. and Harry Gwirtsman, M.D. No group differences were found.

B. Computed Tomography (CT)

CT scans were completed on 11 unrelated affective disorder patients (all with a family history of affective disorder) from the outpatient clinic. These were compared with 26 schizophrenic patients and 20 NIH controls. Despite previous published reports of enlarged cerebral ventricles in patients with affective disorders, none of these patients had ventricular size greater than 1 standard deviation above the control mean. These patients had significantly smaller mean ventricular size than the schizophrenic sample. This work is continuing in order to obtain a larger group of affective disorder patients for comparison.

C. Familial risk and biologic parameters

A family history-biologic parameter study is also underway. In this work morbid risk of illness in relatives will be the dependent variable, and multiple biologic factors (e.g., REM induction response, peptide levels in CSF) will be examined as independent variables to answer the question: Do particular biochemical characteristics correlate with increased genetic vulnerability to affective illness? The data base for this is our family study data, the family data assembled on present and former clinic patients, and biologic measurements accumulated over the past seven years.

2. Studies of the Course and Treatment of Affective Illness

A. Rapid cycling

A family study of rapid cycling bipolar illness is in progress. Patients who are rapid-cyclers are being identified from a pool of several hundred present and former clinic patients and the one hundred bipolar probands from our previous family study. Morbid risk of affective illness in general, and rapid cycling in particular, will be compared in relatives.

B. EB virus (Dr. DeLisi)

It has recently been reported that Epstein-Barr Virus (EBV), the virus associated with mononucleosis, may cause a chronic syndrome lasting for years characterized by vague malaise and includes depression. It is thought that this syndrome may result from persistence of EBV in active form. We therefore have begun to screen all available patients in the NIMH outpatient affective disorders clinic for elevations in antibody titres to EBV capsid and early antigens. The anti-early antigen is of particular interest since this antibody is thought to only be produced during active infection.

Twenty-one patients have had titres determined to date. Four of these had EBV-capsid antibody elevations ($\geq$ 1: 640) and had positive early antigen antibody titres. These evaluations are continuing.

C. Phenylethylamine (PEA) (Dr. DeLisi)

Phenylethylamine has been proposed to be an endogenously produced amphetamine-like psychotomimetic agent. Previous reports have shown that a subgroup of depressed patients, all females, have elevated urinary PEA excretion. Dr. M. Linnoila and colleagues reported dramatic improvement of psychotic symptoms in one of these patients with carbidopa, a peripheral decarboxylase inhibitor. Since carbidopa inhibits the decarboxylation of phenylalanine and the production of PEA, if PEA is associated with psychotic symptoms, these should improve with carbidopa treatment. We therefore have had a protocol for the treatment of psychiatric patients with elevated PEA excretion with carbidopa.

Only one chronically depressed female outpatient, who has been refractory to conventional antidepressant medications has been given a trial of this medication over the past year. No improvement was noted in her depressive symptoms during an approximate 2 month trial. The protocol was discontinued when her depression worsened. In order to find other suitable patients for this protocol, 24 hour urines were collected from 20 available female patients in the outpatient clinic and 8 female controls. Three of these patients and 2 of the controls had 24 hour PEA excretions greater than the range of all previously published normals (<25 micrograms/24 hr.). None of the patients have yet been symptomatic or available for a carbidopa trial.

In order to further explore the nature of the gender difference in PEA excretion, 6 female NIH hospital worker volunteers collected 24 hour urines for approximately 2 months. PEA, MHPG, DOPAC, and VMA were quantified on these samples. Serial plasma estradiol and progesterone concentrations were also obtained. These women had highly fluctuating PEA excretion, that did not conform to any cyclic pattern, nor to specific times of the menstrual cycle. Four of the 6 had at least one 24 hour value that was above the control range. All women had structured psychiatric interviews and RDC lifetime diagnoses were made. Two had lifetime diagnoses of major depression and depressive personality disorder, respectively. One had previous episodes of a major anxiety disorder, although PEA excretion rates were not different in these 2 women compared with the others. No correlation was found with any of the above measured metabolites or hormones. These studies suggest that elevated PEA excretion may not be specific to depression, but may be a normal physiological response. Why it only appears in women, and the mechanism for its production needs to be examined further.

D. Bupropion and carbamazepine treatments

We and collaborators in the Clinical Psychiobiology Branch have treated 20 patients with bupropion. We have also treated 14 persons with the combination of lithium and carbamazepine and collaborators in the Biological Psychiatry Branch have treated others. We are presently

examining this data to identify clinical correlates of bupropion and carbamazepine response.

E. Cognitive abnormalities in affective patients.

Cognitive changes in affective illness have been associated with right-hemisphere abnormalities and global cognitive impairment. Linda Sapin, guest worker has hypothesized that these changes are related to anomalies in handling certain types of information processing strategies rather than overall cognitive impairment or hemospheric dysfunction. We tested 20 normal volunteers and 20 euthymic bipolar patients on a number of parameters assessing relative hemisphere function, cognitive efficiency and analytic vs. synthetic/holistic information processing skills. We found no significant differences between groups in general cognitive ability, and our data did not support the presence of global right hemisphere dysfunction in patients. There were significant impairments, however, in patients' abilities to utilize synthetic encoding strategies (as measured by facial recognition tasks). We conclude that, at least in part, cognitive changes in affective illness are mediated by information processing functions.

3. High Risk Study of Affective Disorder

A group of 58 young people with a manic depressive parent has been assembled along with 29 age-matched controls. In each case the young person has been screened with a structured interview (SADS-L), both parents have been interviewed, and one parent has been asked to fill out a childhood symptom inventory. A careful assessment of life events and social support has been carried out yearly under the direction of Dr. David Pellegrini. One year followup studies have been completed and several subjects have already become ill. Each subject has filled out the Zuckerman Sensation Seeking Scale, the General Behavior Inventory, and the SCL-90. These data are being analyzed. A study of electrodermal response with this group has begun in collaboration with Dr. Ted Zahn. Studies of melatonin sensitivity should begin soon. A study of REM induction may begin following the completion of our replication study of this response (see Z01 MH 00085-08 CNG).

Significance to Biomedical Research and the Program of the Institute

The marker studies are aimed at uncovering indicators of genetic vulnerability as discussed in Z01 MH 00085-09 CNG. These might then lead us to a better understanding of the etiology of these conditions and enable early identification and monitoring of vulnerable persons.

The high risk study may provide confirmatory evidence for biochemical hypotheses regarding the etiology of manic-depressive illness. In addition we will be in a position to assess the interaction of psychosocial variables with genetic vulnerabililty. If a particular genetic vulnerability factor or factors can be demonstrated, new treatment strategies may be

designed. In addition, clinical tools for the early identification of persons vulnerable to affective illness may be forthcoming. Presently available pharmacologic or psychosocial interventions might then be utilized to prevent the social deterioration that may result from untreated affective disorder.

The pharmacologic treatment trials are directed toward the solution of a difficult clinical problem as well as an improved neurochemical understanding of the switch process in bipolar illness.

Our longitudinal followup study of young people (age 15-25) with a manic depressive parent continues. We know that the chances of these "high risk" offspring developing major affective disorder is 25% or more, and that this age is when onset is most likely through the prospective design. We are hopeful of identifying predictive variables that will be understanding the etiology and planning the treatment of affective illness.

Proposed Course of Study

CSF variables are to be correlated with clinical variables in the patient population. A new study of peptide levels in CSF following physostigmine infusion (Z01 MH 00085-08 CNG) is underway.

The high risk study is to continue as planned with further biologic measurements to be made as indicated above.

A study of clinical correlates of carbamazepine and bupropion response in bipolar illness is planned.

The study of cognitive strategies may be extended to patients on lithium and high risk offspring.

Publications:

Nurnberger, J.I., Jr.: Single case study: Diuretic-induced lithium toxicity presenting as mania. J. Nerv. Ment. Dis., in press.

Pons, L., Nurnberger, J.I., Jr., and Murphy, D.: Mood-independent aberrancies in associative processes in bipolar affective disorder: An apparent stabilizing effect of lithium. Psychiatric Res., in press.

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Berrettini, W.H., Nurnberger, J.I., Jr., Hotvedt, P., Simmons-Alling, S., Gershon, E.S.: Vasoactive intestinal peptide and bipolar affective illness: Evidence for an effect of lithium. J. Aff. Dis. 8: 55-59, 1985.

Berrettini, W.H., Rubinow, D.R., Nurnberger, J.I., Jr., Gershon, E.S., and Post R.M.: CSF Substance P in affective illness. Biol. Psychiatry, in press.

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Berrettini, W.H., Goldin. L. R, Nurnberger, J.I., Jr., Gershon, E.S. Genetic Factors in affective illness J. Psych. Res. 18: 329-350, 1984.

DeLisi, L.E., Murphy, D.L., Karoum, F., Targum, S.D., Wyatt, R.J. Phenylethylamine excretion in depression. Psychiatry Res. 13: 193-201, 1984.

DeLisi, L.E., Murphy, D.L., Hamilton, J., Goldin, L.R., Karoum, F., Wyatt, R.J. Elevated phenylethylamine excretion in women. In Bieck, P., (ed.). Proceedings of the Second Trace Amines Symposium, Humana Press, Inc., N.J., in press.

Berrettini, W.H., Nurnberger, J.I., Jr., Gold, P.W., Chretien, M., Chrousos, G.P., Chan, J.S.D., Goldin, L.R., Gershon, E.S.: Neuropeptides in human cerebrospinal fluid. Life Sci., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02236-01 CNG

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Schizophrenia Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	L. DeLisi	Staff Psychiatrist	CNG	NIMH
Others:	E.S. Gershon	Chief	CNG	NIMH
	S. Simmons-Alling	Clinical Nurse Expert	CC	NIH
	W.H. Berrettini	Staff Psychiatrist	CNG	NIMH
	C.W. Dingman	Director	Chestnut Lodge	
	M. Harrington	Visiting Fellow	CNG	NIMH

COOPERATING UNITS (if any)

CC; CHP, LCB, NIMH; NCI; Washington University of St. Louis; Chestnut Lodge; USSR Academy of Medical Science; University of South Carolina

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.4	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A family study of biologic factors in schizophrenia has been initiated. Diagnostic evaluations on 12 families with more than one schizophrenic sibling, and the following biological studies, have been done:

Computed Tomography (CT) was performed on ill and well siblings. Ventricular size was found to have a familial component and also to be significantly larger in the schizophrenic siblings compared with their well siblings and controls.

HLA and other protein markers (immunoglobulin polymorphisms) were determined and linkage analysis performed. There was no linkage to the HLA or Gm loci in these families. Serum immunoglobulin levels (IgG, IgA, IgM) were found to have a familial component, but lower levels did not segregate with illness in families. Abnormal leukocyte counts, atypical lymphocytes, antinuclear antibodies, and antithymic antibodies were not present at an increased frequency in schizophrenics versus their family members and controls.

Herpes simplex, cytomegalovirus and Epstein-Barr viral antibodies were determined in serum of all evaluated subjects. No differences in herpes simplex or cytomegalovirus antibody titres were found among patients and controls. Schizophrenics had significantly higher antibody titres against the Epstein-Barr early antigen than hospital staff members or other controls.

No chromosomal abnormalities, including fragile X chromosome, were found in any of our patients.

Other Investigators (Continued)

E. Hibbs	Psychologist	CHP	NIMH
E. Maxwell	Research Social Worker	CNG	NIMH
L. Goldin	Senior Staff Fellow	CNG	NIMH
J. Nurnberger	Medical Officer	CNG	NIMH
S. Detera-Wadleigh	Senior Staff Fellow	CNG	NIMH
H. Fudenberg	Department Chairman,	Univ. of S.C.	
G. Rougon	Visiting Fellow	LCB	NIMH
P. Sarin	Chief, LTCB		NCI
R. Todd	Professor	Wash. U. of St. Louis	
G. Kolyaskina	Professor, USSR Acad. of Medical Sci.		

Project Description:

We maintain a continual evaluative system to screen by stuctured interview and chart review consecutive admissions to Chestnut Lodge, the Montgomery County catchment area of Springfield Hospital, and community referrals for chronic schizophrenia and for families with more than one schizophrenic member (multiplex families). All first degree relatives, as well as the proband are evaluated by personal interview and medical record review. The Chestnut Lodge and Springfield group serve as probands for a family study of schizophrenia, while a subgroup of these, as well as the multiplex families, have been subjects for biological genetic studies.

Outpatient Clinic for the Treatment of Schizophrenia:

An outpatient clinic was established for pharmacologic treatment of schizophrenic patients who are members of multiplex families. Twelve patients receive treatment as part of this clinic at present. Evaluations of other potential participants and their families are in progress.

Family Study of Schizophrenia:

Structured diagnostic interview procedures are in use for controlled family study of schizophrenia. The Schedule for Affective Disorders and Schizophrenia (SADS) is used to make major psychiatric diagnoses using a modification of the Research Diagnostic Criteria (RDC). The Structured Interview for the DSM-III Personality Disorders (SIDP) is used to determine the presence of DSM-III personality disorders. Family history is obtained on each family member about each member, obstetrical-prenatal histories, pre-morbid social adjustment and the quantification of positive and negative symptom clusters are also obtained. In addition, all psychiatric records are obtained on all subjects for aid in diagnosis.

At present we have reviewed approximately 50 records from Springfield hospital, and have obtained 22 probands with the diagnosis of schizophrenia for family studies. Evaluations have been completed on 26 of their first degree relatives. Seventeen probands have been evaluated from Chestnut Lodge, and 30 first degree relatives have had evaluations completed. Twenty-eight age and gender matched controls and 54 of their first degree relatives have had evaluations completed.

Multiplex Family Studies:

Clinical evaluations have been completed on 18 schizophrenics from multiplex families, 71 of their first degree relatives, and second degree relatives of 3 patients. These studies have included protein markers (HLA and Gm polymorhisms), DNA polymorphisms, autoantibodies, viral-specific antibodies, and structural brain differences on CT scans.

HLA antigens:

HLA (Histocompatibility antigens) linkage analysis has been completed in collaboration with Dr. Goldin and the NIH Blood Bank. HLA halpotypes (A, B, C and Dr) were determined for all first degree relatives in 10 multiplex families (N = 53). Linkage of HLA to schizophrenia was tested using the computer program LIPED. Lod scores were consistently negative, with -2.46 the least negative score at zero recombination (under the additive transmission model.) This rules out close linkage of HLA to schizophrenia.

Computed Tomographic (CT) studies:

Brain lateral ventricular size was quantified in 26 schizophrenic subjects from 12 multiplex families, 10 well siblings, and 20 non-psychotic controls. The mean area of the frontal horns of the lateral ventricles was significantly greater in the schizophrenics compared with their well siblings and controls. In addition, multivariate analysis of the data showed a significant familial component to ventricular size. Although past history of head injury and birth complications were also associated with ventricular size, these were not sufficient to explain either the familial aspect to ventricular size or the association of greater ventricular size with schizophrenia in these families.

Ventricular/Brain Ratios (± S.D.) from 2 CT Scan Slice

	FRONTAL HORNS	BODY OF LATERAL VENTRICLES
SCHIZOPHRENIC SIBLINGS (N=26)	2.81 ± 1.3*	8.14 ± 3.3*
WELL SIBLINGS (N=10)	1.82 ± 0.8	7.39 ± 2.4
NORMAL VOLUNTEERS (N=20)	2.09 ± 1.1	6.14 ± 4.0
AFFECTIVE DISORDER OUT-PATIENTS (N=11)	2.04 ± 0.7	7.31 ± 3.3

*P<.05 (2-tailed t-test) compared with normal volunteers.

2-way Anlaysis of Variance for Ventricular Size (VBR's)
Schizophrenic vs. Well Sibs from 11 Families

	FRONTAL HORNS		BODY OF LATERAL VENTRICLES	
SOURCE OF VARIATION*	F RATIO	p VALUE	F RATIO	p VALUE
FAMILY EFFECT	2.26	.05	2.53	.03
ILLNESS STATUS	4.71	.04	0.38	.54

*Interactions between the two effects were not significant.

This study is designed to determine the relationship of the finding of enlarged brain lateral ventricles in schizophrenic patients to familial schizophrenia. We have concluded from this study that increased ventricular size is present in schizophrenic patients who have a familial component to their illness, and that ventricular size has both a familial component and a relationship to illness in these families. Thus, increased ventricular size fits the requirements for a genetic vulnerability marker but secondary effects of illness or treatment are not ruled out.

Positron Emission Tomography (PET):

The severity of symptoms and the therapeutic effect of pharmacologic treatment was not associated with relative frontal metabolic rates. Activity in temporal cortex of schizophrenic and euthymic depressed patients was found to be elevated from control values, but basal ganglia activity was not different from controls. These results may suggest some anatomical localization of abnormal brain functioning in schizophrenia that should be further explored.

Immunologic Dysfunction:

Some schizophrenic patients have abnormalities in immune system functioning. We previously reported decreased immunoglobulin production, increased percentages of T suppressor and B lymphocytes, decreased natural killer cell activity of lymphocytes, and increased autoantibody production in subgroups of schizophrenic patients. Over the past year further pursuit of these findings has included:

1. Immunoglobulin Concentrations: All available members of 11 multiplex families had serum immunoglobulin IgG, IgA, and IgM levels determined. In addition, approximately 25 unrelated chronic schizophrenic patients (both inpatients and outpatients) had immunoglobulin levels determined in serum, and CSF studies are in progress.

Preliminary analysis revealed a significant familial component to all serum immunoglobulin concentrations, and significantly higher IgM concentrations in females compared with males. There was, however, no segregation of low levels of any of the immunoglobulins with schizophrenia in any of the multiplex families studied, nor were mean immunoglobulin concentrations lower in this sample of patients than controls.

2. Gm Immunoglobulin Polymorphisms: Eight multiplex families, 10 schizophrenic sibling pairs, 25 unrelated schizophrenics, and 25 controls had serum samples analyzed for Gm haplotypes by Dr. Hugh Fudenberg. Linkage and association analysis is in progress.

3. Autoantibody Production: We have previously reported that approximately 20% of schizophrenic patients had elevated serum antinuclear antibody titres. In another study, we reported a smaller percentage of patients had evidence of antibrain antibody production. In the multiplex families, 2 well relatives, but none of the schizophrenics, had elevated antinuclear antibody titres.

Ten unrelated patients and 10 controls had serum samples examined for antibrain antibodies using immunoradiography (Dr. G. Rougon). Since a similar percentage of patients and controls had evidence of antibrain antibodies with this method, we have not pursued this further.

Increased antithymic antibody production has been reported in schizophrenic patients (G. Kolyaskina). Antithymic antibodies (antibodies to proteins on the surface of lymphocytes), if excessively produced, could explain some of the immune system abnormalities previously reported. Since they are also known to cross-react with brain antigens, antithymic antibodies may be similar to the antibrain antibodies that we, and other investigators, have found in schizophrenic patients. Therefore, samples from 10 multiplex families, 23 unrelated schizophrenics and 15 controls had antithymic antibody determinations (Dr. Kolyaskina). Preliminary analysis of the data reveals no significant differences between these groups.

Dr. Richard Todd recently reported an association between antibodies to serotonin receptors and infantile autism. Since both autistic children and chronic schizophrenic patients have been reported to have elevated blood serotonin levels, and localized brain serotonin elevations (postmortem), a collaboration is in progress with Dr. Todd to determine whether schizophrenic patients have antibodies to serotonin receptors.

4. Viral-specific Antibody Production:

A viral hypothesis for the development of schizophrenia has been proposed by several investigators. While no association with one specific type of virus has been established, some investigators have focused on the herpes class of DNA viruses, while another researcher has proposed a retroviral etiology.

To explore whether retroviral infection may be present in some schizophrenic patients, we used an assay for antibodies to reverse transcriptase and Human T-cell Leukemia Virus (HTLV; the only group of retroviruses known to infect humans). Since all retroviruses produce reverse transcriptase, antibodies produced against it would be an indication of some retrovirus infection. Sera from 15 chronic schizophrenic patients were compared with sera from 15 controls for presence of these antibodies. No differences were noted.

Antibodies to herpes simplex (HSV-1, HSV-2), cytomegalovirus (CMV), and Epstein-Barr virus (EBV-VCA, EBV-EA) were determined in sera from 38 unrelated chronic schizophrenic patients, 12 multiplex families, and 2 control groups. The distribution of antibody titres to herpes simplex and cytomegalovirus were similar among all groups. Approximately 30% of the schizophrenics and 33% of Chestnut Lodge staff members (N=24) had antibody titres to EBV-VCA that were significantly higher than a normal volunteer control sample (N=17) and a general population (40 pre-marital blood samples). Twenty-seven of the unrelated schizophrenic patients and all of the Chestnut Lodge staff members had EBV-EA antibody titres determined. Significantly more of the schizophrenic patients (17 of 27) than hospital worker controls (7 of 24) had positive anti-EBV titres ($chi^{-2} = 5.8$; $p < .85$). Since elevated EBV-EA may suggest persistent EBV infection, we are further exploring this finding.

5. Acyclovir Protocol

Acyclovir, an antiviral agent that specifically inhibits herpes and EBV viral DNA polymerase, is being administered in a double blind trial to patients with increased antibodies to EBV. Six patients are currently in this protocol.

Cytogenetic Studies:

Chromosome analysis of 24 unrelated male schizophrenic patients were completed in order to determine if increased frequencies of any chromosomal aberrations, including the fragile X syndrome, exist in this population None of these patients were found to have any chromosome abnormalities or a fragile X chromosome. This study is still in progress.

Molecular Genetics:

DNA obtained from whole blood from the previously mentioned unrelated schizophrenic populations, multiplex nuclear families, and two extended pedigrees have been, and continue to be, obtained for association and linkage studies (Dr. S. Detera-Wadleigh). Several cDNA probes for neuropeptides relevant to several of the biochemical hypotheses of schizophrenia, as well as other probes that serve as specific chromosomal markers, have been used in these studies (see Z01 MH 02237-01 CNG and Z01 MH 00084-11 CNG). In addition, lymphocytes from 2 extended pedigrees with more than 2 schizophrenic members have been placed in culture in a cell repository in order that DNA from these individuals will be continually available for future studies.

CSF Studies:

Collection of CSF samples from the above patients (sib pairs and unrelated patients) are in progress in order to detect the presence of abnormal protein production in the CSF (in collaboration with Drs. Berrettini and Harrington, see Z01 MH 00084-11 CNG and Z01 MH 02238-01).

Significance to Biomedical Research and the Program of the Institute

A genetic susceptibility for the development of schizophrenia has been implied by previous family, twin, and adoption studies, although the inherited biologic factors are unknown. The extent of other psychopathology and clinical syndromes additionally related to the inheritance of schizophrenia is not yet known. Our diagnostic family interview study, now in progress, will distinguish the heritable diagnostic components of this disorder. These probands and families additionally form a population for the examination of the association of several putative biological markers with schizophrenia.

Our growing registry of families with at least 2 living schizophrenic first degree relatives is a valuable resource to test for an association of inherited biological markers to the inheritance of schizophrenia. The value of this group of families is enhanced by our maintenance of lymphoblast cell lines from families in this group that appear most informative for linkage analyses. As new DNA markers become available, this will be a continual, valuable source for schizophrenic genetic marker studies.

Proposed course of the Project:

We plan to continue the above investigations and expand our samples of schizophrenic probands and multiplex families for further biological marker studies, focusing on the molecular genetic approach, but also using this population to test the significance of other biologic factors that have been associated with schizophrenia.

Publications

DeLisi, L.E., Mirsky, A.F., Buchsbaum, M.S., van Kammen, D.P., Berman, K.F., Caton, C., Kafka, M.S., Ninan, P.T., Phelps, B.H., Karoum, F., Ko, G.N., Korpi, E.R., Linnoila, M., Sheinan, M. and Wyatt, R.J.: The genain quadruplets: A diagnostic and biochemical follow-up. Psychiatry Res. 13: 59-76, 1984.

Buchsbaum, M.S., DeLisi, L.E., Holcomb, H.H., Cappelletti, J., King, A.C., Johnson, J., Hazlett, E., Dowling-Zimmerman, S., Post, R.M., Morihisa, J., Carpenter, W., Cohen, R., Pickar, D., Weinberger, D.R., Margolin, R. and Kessler, R.M. Anteroposterior gradients in cerebral glucose use in schizophrenia and affective disorders. Arch. Gen. Psychiatry 41: 1159-1168, 1984.

DeLisi, L.E., Buchsbaum, M.S. and Holcomb, H.H.: Increased temporal lobe glucose utilization in schizophrenia. Biol. Psychiatry, in press.

DeLisi, L.E., Buchsbaum, M.S., Holcomb, H.H., Dowling-Zimmerman, S., Pickar, D., Boronow, J., Morihisa, J.M., van Kammen, D.P., Carpenter, W., Kessler, R., and Cohen, R.M.: Lack of clinical correlates to hypofrontality on PET in schizophrenia. Am. J. Psychiatry 142: 78-81, 1985.

DeLisi, L.E.: Immunologic studies in psychiatric disorders. In Kurstak, E. (Ed.): Viruses, Immunity, and Mental Illness. Plenum Publishing Corp., in press.

DeLisi, L.E. and Crow, T.J.: The viral and immune dysfunction hypotheses of schizophrenia. In Roy, A. (Ed.): The Biology of Schizophrenia: Psychiatric Clinics of North America. W.B. Saunders Co. Philadelphia, in press.

DeLisi, L.E. and Sarin, P.: Lack of evidence for retrovirus infection in schizophrenia. Br. J. Psychiatry, in press.

Nurnberger, J.I., Goldin, L. R., DeLisi, L.E., Gershon, E.S.: The Genetics of Schizophrenia. Procedings of the (CINP Satellite Symposium: Schizophrenia, An Integrative View. Milan, Italy, June, 1984 (in press).

DeLisi, L.E., Goldin, L., Hamovit, J.R., Maxwell, M.E., Kurtz, D., Gershon, E.S.: Is cerebral ventricular enlargement a genetic marker for schizophrenia? Psychopharm. Bull., in press, 1985.

Buchsbaum, M.S., DeLisi, L.E., Holcomb, H.H., Hazlet, E., Kessler, R.: Cerebral glucography in schizophrenia. In Greitz, T., Ingvar, D.H., Widen, L. (Eds): Metabolism of The Human Brain Studied With Positron Emission Tomography. Raven Press, New York, pp. 471-484.

DeLisi, L.E., Goldin, L.R., Gershon, E.S., Nasrallah, H.A.: Subgroups in schizophrenia. The Lancet (letter to the editor), in press.

DeLisi, L.E., King, A.K., Targum, S.: Serum immunoglobulin concentrations in patients admitted to an acute psychiatric inpatient service. Brit. J. Psychiatry, 145: 661-664, 1984.

DeLisi, L.E., Murphy, D.L., Karoum, F., Targum, S.D., Wyatt, R.J. Phenylethylamine excretion in depression. Psychiatry Res., 13: 193-201, 1984.

DeLisi, L.E., Weber, R., Pert, C.B.: Anti-brain antibodies in psychotic patients: review and perspectus. Biol. Psychiatry, 20: 110-119, 1985.

DeLisi, L.E., and Wyatt, R.J.: Neurochemical aspects of schizophrenia. In Lajtha, A. (Ed.): Handbook of Neurochemistry, vol. 10, 1984, pp. 553-587.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH 02237-01 CNG

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	S. Detera-Wadleigh	Senior Staff Fellow	CNG, NIMH
Others:	E.S. Gershon	Chief	CNG, NIMH
	J.I. Nurnberger, Jr.	Medical Officer	CNG, NIMH
	W. Berrettini	Staff Psychiatrist	CNG, NIMH
	L. DeLisi	Staff Psychiatrist	CNG, NIMH
	L. Goldin	Senior Staff Fellow	CNG, NIMH

COOPERATING UNITS (if any)

Oxford University; Purdue University; Howard University; Michigan State University; NIADDK

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	2.3	1.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Genomic DNA was extracted from three pedigrees of manic depressive and schizophrenic patients. Similarly, DNA was obtained from a panel of unrelated patients and control individuals. One family with manic depressive illness was informative using the restriction fragment length polymorphisms (RLFP's) in the somatostatin gene. Linkage analysis was performed on this pedigree under several modes of inheritance. The lod scores were negative, thus close linkage of the somatostatin region to the disease locus is unlikely. No apparent association was found with this gene. All manic-depressive families were informative using the insulin probe. Examination of the insulin gene data for linkage awaits blotting of completed pedigrees.

Novel RFLP's were discovered using complement C4, neuropeptide Y and the β-hexosaminidase α-chain as cDNA probes. Initial results with neuropeptide Y revealed the presence of an RFLP allele in the DNA of several several manic-depressive and schizophrenic patients but not in any normal individuals.

Calmodulin-specific cDNA clones were isolated from a rat brain λgtll-cDNA library using calmodulin cDNA from Xenopus laevis.

Other Investigators (Continued):

J.R. Hamovit	Research Social Worker	CNG NIMH
M. Carroll	Professor	Oxford University
J.E. Dixon	Professor	Purdue University
C. Minth	Research Associate	Pudue University
F. Friedberg	Professor	Howard University
J.E. Wilson	Professor	Michigan State Univ.
R. Myerowitz	Staff Fellow	NIADDK

Molecular Genetics

I. DNA Polymorphisms in the Genetics of Neuropsychiatric Disorders

Project Description:

Objectives:

Polymorphisms in DNA are generated by a variety of events such as base pair substitutions, deletions, insertions and chromosomal rearrangements. In recent years these polymorphisms have become extremely useful in gene mapping as well as in finding a marker gene that is linked to a disease locus. It is the purpose of this work to exploit this feature to search through the human genome for a major locus for the neuropsychiatric disorders, manic depressive illness and schizophrenia.

Methods Employed:

Genomic DNA is extracted and purified from lymphoblast cell lines or blood samples derived from patients, members of the patients' extended family and normal (control) individuals using standard procedures. The DNA is digested with a variety of restriction enzymes, the fragments fractionated on an agarose gel and eventually transferred to a nylon membrane by Southern blotting. Hybridization of the DNA with a radioactively labelled cDNA probe is done and the membrane is washed. The pattern of the restriction fragments is revealed by autoradiography.

Major Findings:

We have blotted genomic DNA from three pedigrees of manic depressive and schizophrenic patients. Although DNA from other family members are yet to be collected, we proceeded with the analysis of known restriction fragment length polymorphisms (RFLP) in the regions of somatostatin and insulin genes. Cultures of somatostatin and insulin clones were kindly provided by Dr. Graeme Bell, Chiron Corporation, California. Based on the RFLP's generated by the action of BamHI and EcoRI in the somatostatin gene, linkage analysis was performed on one informative family with affective disorder. Results indicate that under several modes of inheritance, close linkage of the somatostatin gene to the disease locus is ruled out as very unlikely. We conclude that the somatostatin area in chromosome 3 can be tentatively excluded as a possible marker for manic depressive illness. Furthermore,

association of affective disorder and schizophrenia with somatostatin was determined using blots containing DNA's of unrelated patients. The result of this analysis was negative. Using the highly polymorphic Pvu II locus in the flanking region of the insulin gene, all the families analyzed were found to be informative. Complete linkage will be done when DNA's of all the members of the pedigrees are blotted.

Novel RFLP's were discovered with four restriction enzymes when the complement C4 cDNA was used as a probe. A clone of C4 was obtained from Dr. Michael Carroll, Department of Biochemistry, University of Oxford, England. Complement C4 maps within the highly polymorphic HLA region on chromosome 6.

Using the β -hexosaminidase α-chain supplied by Dr. R. Myerowitz, polymorphism in DNAs from several schizophrenic patients was observed with one restriction enzyme. Similarly, a new RFLP was observed using a neuropeptide Y cDNA probe, supplied by Drs. Jack E. Dixon and Carolyn Minth, Department of Biochemistry, Purdue University, Indiana. Interestingly, this polymorphism is absent in 14 normal individuals but present in about 16% of both manic depressive and schizophrenic patients. By increasing sample size we should be able to ascertain whether this provocative finding will persist.

II. Molecular Cloning of Rat Calmodulin Gene

Objectives:

The discovery of a pseudogene in chicken calmodulin by A. R. Means' group raises the question of whether mammalian calmodulin is encoded by more than one gene. We plan to investigate this problem by cloning rat calmodulin gene. Furthermore, because of the multifaceted functions of calmodulin, it is important to study gene expression in various structures of the brain during development, aging and certain neurologic conditions.

Methods Employed:

This project is being done in collaboration with Dr. Felix Friedberg, Department of Biochemistry, Howard University, Washington D.C.

A rat brain λgt11-cDNA library, kindly provided by Drs. A. Dossett and L. Fritz, California Institute of Technology is screened for calmodulin cDNA sequence using *Xenopus laevis* calmodulin cDNA from Dr. Igor Dawid, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development. Briefly, the screening procedure includes: infection of Y1090 bacteria with the library, growing on agar plates, transfer of DNA to nitrocellulose after lysis, hybridization of the DNA with radioactively labelled *X.laevis* calmodulin cDNA and autoradiography. Screening is repeated at least three times until calmodulin specific cDNA clones are isolated. These clones are amplified, purified and characterized by restriction mapping and DNA sequencing. The cDNA clones are used to isolate genomic clones from a rat genomic library.

Major Findings:

We have isolated calmodulin specific cDNA clones. The cDNA inserts are now being characterized and subsequently subcloned into M13 for sequencing.

III. Cloning of Brain Hexokinase cDNA

Since last year's report all of the work pertaining to this project have been done in Dr. John E. Wilson's laboratory, Department of Biochemistry, Michigan State University, Michigan. Dr. John E. Wilson is the principal investigator of this project.

Significance to Biomedical Research and the Program of the Institute:

The observed family clustering of affective disorder and schizophrenia strongly suggests a genetic involvement in the transmission of these diseases. However, the absence of a reliable biochemical marker continues to preclude better understanding of the genetics of these conditions. This difficulty can be overcome by utilizing recombinant DNA techniques to identify a polymorphic DNA marker that might be linked to the locus of the disease. In addition, our studies are designed to look for association of the disease with known loci on various chromosomes. A by-product of these studies is the systematic exclusion of various regions on the human genome as potential genetic markers for affective disorder and schizophrenia since different kinds of cDNA probes will be used. Discovery of a DNA marker locus will have an immense impact on patient care as well as on the elucidation of the basic defect in neuropsychiatric disorders.

Calmodulin is a calcium-binding protein that is highly abundant in the brain. It stimulates the activity of many important enzymes such as adenylate cyclase, phosphodiesterase and some kinases. Our attempt to clone and characterize the gene is pertinent to the understanding of the role of calmodulin in development, aging and diseased states.

Proposed Course of Study:

In the succeeding year we plan to examine other cDNA probes for new RFLP's. These polymorphisms will be used to study linkage and association. Collection of DNA from all the pertinent members of the existing pedigrees of specific structural genes with neuropsychiatric disorders will be completed. Sample size for normal controls and unrelated patients will be expanded.

We plan to characterize and determine the detailed structure of rat calmodulin cDNA. We will use the cDNA to isolate genomic clones. The organization of the coding and noncoding sequences will be examined. The cDNA will be used also to study gene expression in rat brains.

Publications:

Detera-Wadleigh, S., Karawya, E., and Wilson, S.H.: Synthesis of DNA Polymerase by *In Vitro* Translation of Calf RNA. *Biochem. Biophys. Res. Commun.* 122: 420-427, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00935-18 CNG

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
*Studies of Plasmids and Small Genomes in Human Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	C.R. Merril	Chief Biochemical Genetics Section	CNGB NIMH
Others:	D. Rath	Staff Biologist	CNG NIMH
	M. Harrington	Visiting Associate	CNG NIMH
	M. Harasewych	Guest Scientist	CNG NIMH
	S. Olson	Guest Scientist	CNG NIMH
	B. Brown	Chief, Forensic Science Research	FBI Academy
	M. Kaiser	Ophthalmology	NEI
	M. Bray	Pathologist	D.C. General Hosp.

COOPERATING UNITS *(if any)* NEI; D.C. General Hospital; Forensic Science Research Group, FBI Academy, Quantico, Virginia

LAB/BRANCH
Clinical Neurogenetics Branch

SECTION
Section on Biochemical Genetics

INSTITUTE AND LOCATION
NIMH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of a normally occurring plasmid genome, the mitochondrial genome, in human brain tissues have revealed that most of the mitochondrial DNA is in an oligomeric form. These oligomers appear to represent dimeric, trimeric, and tetrameric forms of the mitochondrial DNA. Previous studies of the mitochondrial genome in human tissues failed to reveal the predominance of oligomeric forms, since they did not involve human postmitotic tissues, such as the brain tissues examined in the current investigations.

Lack of mtDNA repair mechanisms may account for the relatively high mutation rate found in this genome, as compared to the nuclear or chromosomal genome. A survey of the clinical literature revealed a number of diseases that display non-Mendelian maternal inheritance patterns and may involve the mitochondrial genome. Studies on the primary structure of mitochondrial DNA from normal individuals and from patients with maternally inherited diseases have been initiated. These studies are being performed in collaboration with the FBI Forensic Science Research Group to determine whether these genetically inherited variations in the human mitochondrial genome are sufficient to establish individuality for forensic studies.

*The above title was formerly Effect of Viruses and Plasmids on the Biochemistry of Living Organisms.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description

The presence of DNA within the mitochondrial organelle was noted more than 20 years ago. This mitochondrial DNA (mt DNA) has been shown to represent a unique genome and to occur in a closed circular structure. In higher animals and man this molecule is approximately 5 microns in length , as determined by electron microscopic analysis. Electron microscopy has also revealed additional mt DNA species in a variety of tissues. These "abnormal" forms of mt DNA can be grouped into two categories: the circular dimers or oligomers, which consist of randomly linked mitochondrial genomes; and the catenated oligomeric forms, which are comprised of interlocked circular molecules, resembling links of a chain.

Early investigations into the occurrence of these complex forms of mitochondrial DNA examined primarily neoplastic tissues. Circular dimers were initially discovered in the leukocytes of patients suffering from granulocytic leukemia. Further studies showed that these abnormal forms were virtually absent (less than 1%) in the leukocytes of normal donors and in a variety of normal tissues. On the other hand, electron microscopic analysis has revealed that the catenated forms of mt DNA are found in nearly all tissues and cell lines. A review of published literature indicates that most samples of mt DNA from normal animal tissues or embryos, contain catenated dimers at frequencies of between 4 and 9%. Catenated higher oligomers, mostly trimers, have been detected at frequencies of approximately 1%. Additionally, a study by Piko and Matsumoto (1977) has indicated that the occurrence of these complex forms of mt DNA may change with age. These investigators found an increase in both circular dimers and total complex forms in the tissues, especially the brains, of senescent mice.

This latter finding prompted our study of human mt DNA in relation to the aging process. Mitochondrial DNA was purified from autopsy samples, predominately brain tissue, and studied using agarose electrophoresis. Samples were also digested by restriction endonucleases and analyzed by hybridization with purified mt DNA. In addition to the 16.5 kb band, which represents the mitochondrial genome monomer, four bands of higher molecular weight have been detected on gels. These bands appear to represent dimeric, trimeric, tetrameric and higher oligomeric forms of mitochondrial DNA. The frequencies of these species have been measured by computer densitometry. The dimeric form was found to occur at a frequency of approximately 8.5%, the trimer, 6.0%, the tetramer, 66.0%, and the higher oligomers, 5.0%. These frequencies were determined for a variety of human tissues and showed no significant changes over a wide age range (2 months - 90 years). These results reveal a predominance of complex forms of mt DNA previously undetected by electron microscopy. The significance of these high frequencies of oligomers is currently unknown.

In addition to the 16.5 kb band and the oligomeric bands, several lower molecular weight bands have been observed in mt DNA purified from human brain tissue. These bands appear to fall into two categories:

those which hybridize to mt DNA sequences, and those which show no homology to the mitochondrial genome. Further investigation of these lower molecular weight species is needed for their full characterization.

We are currently in the process of cloning mitochondrial DNA sequences derived from human platelets. These cloned sequences will be used as probes to determine the origin of the small molecular weight bands mentioned previously. In addition, these clones will be used to produce subclones for use in sequencing analysis. We are interested in examining three regions of the mitochondrial genome for sequence variation. These three areas differ in their degree of conservatism: the D-loop region is considered to be most variable, the t-RNA region selected is considered to be highly conserved, while the region coding for cytochrome oxidase subunit II has an intermediate degree of sequence variability. These probes will be used to screen mitochondrial genomes from a number of individuals for these selected sequences. Using DNA sequencing methodologies, we will examine the degree of heterogeneity within and between individuals in order to determine of the rates of mutation and evolution of these portions of the mitochondrial genome. The mutation rate of mitochondrial DNA is known to be relatively high compared to the nuclear genome (by a factor of 5 to 10 times). This high mutation rate is in part due to the lack of both replicative and postreplicative repair mechanisms in the mitochondria.

The rapid rate of base substitution in the mitochondrial genome has prompted investigators to utilize mt DNA in studies of population genetics. Brown and his colleagues extracted mt DNA from placental tissue from 21 individuals of diverse ethnic and geographic origin. The mt DNA was then digested with 18 different restriction endonucleases and analyzed electrophoretically. This limited analysis allowed each of the 21 individuals to be genetically identified. Based upon these findings, we intend to examine the mt DNA from a variety of individuals using sequencing analysis. By determining the nucleic acid sequence of a region of the mitochondrial genome, we believe that we can establish genetic individuality in addition to determining ethnic origin.

This concept has obvious applications to the field of forensic science. The ability to irrefutably associate an individual with samples collected from the scene of a crime could become a powerful tool in criminal investigations. The molecular markers currently used in such investigations can only support other evidence and do not constitute individual identification. Consequently, a collaborative effort has been initiated with the Federal Bureau of Investigation (FBI) to develop methodologies that would utilize the sequence information obtainable from mt DNA for identification purposes.

The primary procedure which needs to be developed for this application is the extraction of mt DNA sequences from very small samples. Currently, we are attempting to bind single stranded mt DNA sequences of interest to inert particles. These particle bound DNA segments will then be hybridized to whole cell DNA extractions from samples obtained from a crime scene (ie., dried blood or semen). After the hybridi-

zation reaction has gone to completion, the entire mixture can be loaded into a micro-column and unhybridized nucleic acids can be washed away. The mt DNA of interest is then eluted by increasing the temperature to 80 or 90°C, cloned and sequenced. This sequence can then be compared against a library of known sequences to determine ethnic origin, and to the suspect's sequence as a form of molecular "fingerprint."

Sequencing technology will also be applied to the examination of disease states to determine if mutations in mt DNA, which is inherited only from the maternal gamete, are associated with maternally inherited diseases. The initial disease chosen for investigation is Leber's optic atrophy. This disease is a hereditary cause of blindness with onset at 13 to 40 years of age. To date, there have been no reported cases of transmission of the disease from an affected male. Affected females pass on the disease to approximately 50% of their offspring, however, an additional 40% of the offspring are carriers of the defect. At the present time, we have clinically studied a pedigree with Leber's optic atrophy in collaboration with Dr. Kaiser (NEI). We have obtained platelets through the NIH Blood Bank from normal controls and an affected individual from this pedigree and have extracted the mt DNA. We are presently beginning sequence analysis of the mitochondrial genome of this patient.

An additional approach to investigating the mitochondria's role in various disease states is to examine the proteins coded for by the mitochondrial genome. Currently, we are developing the methodology for an in vitro translation assay for mitochondrially coded proteins. Patient samples will then be screened, using two-dimensional gel electrophoresis, to detect alterations in these proteins in the various diseases with suspected mitochondrial associations.

This project also examines the extent of viral integration into the human genome. Viral probes will be used to screen cellular DNA from extensive pedigrees to determine if integrated viral sequences are inherited in a Mendelian manner. Seventy fibroblast cell lines have been established from individuals belonging to a family with histologically confirmed Alzheimer's disease. We are currently selecting the viral probes to be used in this study based upon the likelihood of original virus infection and the possibility of integration.

In addition to determining the inheritance pattern of these viral sequences, association with the disease state will also be examined. To investigate the genetic linkage between the integrated viral sequences and disease loci, the DNA from several diseases including Alzheimer's disease and neuropsychiatric disorders such as schizophrenia, will be analyzed. Samples from individuals to be included in this study are presently being collected.

Significance to Biomedical Research

The relative abundance of the various mitochondrial genomic forms may provide an indication of the presence of a diseased state, such as neoplasia, or to the aging condition of the organism. Previous research has shown that the frequencies of complex forms of mt DNA increase in both of these conditions. Sequencing of the mitochondrial genome may

pinpoint the precise nucleic acid mutation responsible for some of the maternally inherited or mitochondrially associated diseases. In turn, this would provide molecular markers for determining carriers of diseases such as Leber's optic atrophy. It is also possible that in some conditions, alterations in mitochondrial transcriptional or translational controls may result in a disease state. We intend to investigate this possibility with an in-vitro translation assay to determine which proteins are mitochondrially encoded. Patient samples will then be screened using two dimensional electrophoresis to monitor changes in patterns and concentrations of these proteins.

Analyses of the inheritance patterns of integrated viral sequences may lead to the discovery of genetic linkage between these nucleic acid segments and disease loci. Such findings could eventually provide molecular probes for investigations into the pathogenesis of diseases, including Alzheimer's disease and schizophrenia.

Proposed Course of Research

We plan to continue our investigations into the role of the mitochondrial genome in aging and disease. The mitochondrial DNA of various individuals will be sequenced to determine: the heterogeneity of the mitochondrial nucleic acid within and between individuals as an indication of the mutation rate, the potential of mt DNA sequences for determination of ethnic origin and individual identity, and the relationship between mutational events and disease states. We also intend to screen a group of individuals with certain maternally inherited disorders to determine if there is a relationship between these diseases and mitochondrial protein patterns and concentrations.

This work will also utilize tissues and cell lines established from large pedigrees with Alzheimer's disease and other familial disorders to survey for viral sequences in the nuclear genome. The precise nature of the integrated viruses will be examined as will as their potential disease associations. If this approach is promising, it will be extended to investigate other neuropsychiatric disorders.

Publications

1. Merril, C.R., and Harrington, M.G.: The Search for mitochondrial inheritance of human disease. Trends in Genetics. 1:140-144, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00941-05 CNG

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Genetics and Metabolic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	C.R. Merril	Chief, Biochemical Genetics Section	CNG, NIMH
Others:	M. Harrington	Visiting Associate	CNG, NIMH
	M. Harasewych	Guest Scientist	CNG, NIMH
	S. Olson	Guest Scientist	CNGB, NIMH
	D. Goldman	Chief, Unit on Molecular Genetics	LCS, NIAAA
	R.S. Burns	Associate Professor	Vanderbilt Univ.

COOPERATING UNITS *(if any)*

NIAA; NINCDS; NINCHD; DCRT; St. Elizabeth's Hospital; Johns Hopkins University Hospital; University of Southern Illinois; University of Alabama School of Medicine; Vanderbilt University; USUHS

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Biochemical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Twenty-seven independent polymorphic protein loci including 2 very recently identified were detected by two-dimensional electrophoresis of serum, erythrocyte, and fibroblast samples from individuals in two large pedigrees. The genetic basis of these protein variants was corroborated by their mendelian inheritance patterns, and by their quantitative gene dosage dependence. These protein polymorphisms should prove useful for genetic screening of families with genetic diseases.

Disease associated proteins (apparent pI 5.1 and 5.2, and MW of 29k and 26k, respectively) have been found in the cerebrospinal fluid of 21 patients (100%) with Creutzfeldt-Jakob disease (CJD). Of 18 other CNS diseases studied only spinal fluid from herpes simplex encephalitis (HSE) patients (50%) displayed similar proteins. Two other disease associated proteins (apparent pI 5.7 and 5.9, with a MW of 40k for both proteins) have been observed in the spinal fluids of patients with: CJD (67%), HSE (32%), multiple sclerosis (13%), Parkinson's Disease (12%), and schizophrenia (32%). Observation of these disease associated and polymorphic proteins was facilitated by the use of sensitive silver staining methods, introduced by this section, for the detection and quantitation of proteins separated on polyacrylamide gels. These staining methods continue to be refined, with sensitivity currently at the .01 ng level.

Collaborators:

L. Goldin	Senior Staff	CNG	NIMH
D. Asher	Senior Staff	CNSS	NINCDS
D.C. Gajduseck	Chief,Lab.of Central Nerv.Sys.Studies	CNSS	NINCDS
D. McFarlin	Chief, Neuroimmunology Branch	NI	NINCDS
D. Price	Chairman & Professor Pathology	Johns Hopkins Univ	
L. Cork	Professor of Pathology	Johns Hopkins Univ	
T. Sunderland	Staff Fellow		NIMH
W. Berrittini	Staff Psychiatrist	LCN	NIMH
J. Nurnberger	Staff Psychiatrist	LCN	NIMH
E. Gershon	Chief, Clinical Neurogenetics Branch		NIMH
P. Martin	Clinical Director		NIAAA
R. Garruto	Staff Scientist		NINCDS
P. Brown	Neurologist		NINCDS
E.F. Torrey	Staff Psychiatrist St Elizabeth's Hosp., Wash.,DC		
R. Polinsky	Psychiatrist		NINCDS
M. Ebert	Chairman, Dept of Psychiatry, Vanderbilt Univ		
P. Voisin	Visiting Fellow		NICHD
D. Klein	Senior Scientist		NICHD
B. Manyam	Neurologist Univ of Southern Illinois		
F. Lakeman	Research Scientist Univ of Alabama Schl of Med		
I. Hay	Professor of Virology		
M. Dalakos	Neurologist		USUHS
G. Campbell	Senior Staff		DCRT

Project Description

1. Objectives

This project involves the study of the effects of gene and protein alterations in normal persons as well as in a spectrum of diseases of the nervous system. This broad approach recognizes the need to identify alterations in the context of frequently heterogenous clinical characterizations. Two approaches are being used based on the methodological developments and research progress of this laboratory in recent years. One utilizes the 35 mendelian polymorphic proteins identified by two dimensional electrophoresis (2DE) for genetic screening of families with a known genetic disease in a quest for linkage. The other approach involves the identification of physiological protein alterations and proteins associated with pathological processes. These broad survey methods employed in the initial screening are especially necessary to provide a biochemical baseline from which to characterize the poorly defined chemical basis of many neurological and psychiatric disorders. From such a baseline of protein measurements, fluctuations from normal may then be scrutinized with further state-of-the art biotechnology. Abnormal proteins may,for example, be purified and molecular probes made to determine their origin.

2. Methods Employed

Subjects:

Human tissues and fluids have been collected from rigorously defined clinical and normal populations utilizing the expertise of various collaborators from within NIMH and from outside medical scientific communities.

Laboratory Procedures:

These include tissue culture, radioactive protein pulse-labelling, protein one-and two-dimensional electrophoresis, protein electroblotting and immunoprobe analysis, computer assisted densitometry, and studies of viral interactions with cultured cells.

Protein detection techniques have been specially developed in this laboratory to enable detection of trace proteins in biological samples, such as cerebrospinal fluid (CSF). These methods continue to be developed for sensitive detection with varied specificity. Silver stains, first developed for protein detection after polyacrylamide electrophoresis in this laboratory, have been mainly utilized in recent years. Most proteins have been detected with this chemically-developed silver stain process at a lower limit of sensitivity of one nanogram, but this has been improved to 0.01 nanogram and can also now be used to detect proteins on nitrocellulose.

In order to determine other features of proteins, such as tracemetal content and the degree of phosphorylation, two new methods have been developed for postelectrophoretic study. These methods, electron microprobe analysis and neutron activation followed by autoradiography, are capable of semi-quantitative analysis in the nanogram range.

This laboratory, in conjunction with other NIMH collaborators, has developed the use of computer assisted densitometry for quantitation of protein separated by 2DE. These methods continue to be refined, particularly to enable the optimal use of the enormous data-sets from experiments with 2DE. In the past year, dataset analyses have been improved with use of varied Statistical Analysis Systems and BMDP Programs that are available from the DCRT.

3. Major findings

1) Genetic Polymorphic Studies

Collaborative studies of polymorphic proteins by two dimensional gel electrophoresis have revealed 7 serum, 4 erythrocyte and 17 fibroblast protein polymorphisms in a survey of individuals belonging to two large families. These polymorphic proteins were discovered by their charge variation on electrophoretic gels, and their polymorphic nature confirmed by their mendelian transmission in the two families and by their gene dosage effect, observed by quantitative comparisons of these proteins in homozygous and heterozygous individuals. Linkage analysis in collaboration

with Dr. Lynn Goldin (see report Z01 MH 00084-11 CNG) revealed that each polymorphic protein represents a product of an independent locus, with the single exception that the erythrocyte locus (RBC4) appears to be identical to the fibroblast locus (NC27). Two of the polymorphic proteins were positively identified as glyoxalase-1 and phosphoglucomutase-3 by linkage studies. Two other proteins were shown to be linked to apolipoprotein E and alpha-1 haptoglobin. These studies indicate a minimum level of average protein charge heterozygosity of 2.2% for human cellular proteins and 5.6% for serum proteins.

2) Physiological and Pathological Protein Studies

A. CSF Studies: Initial studies of a wide variety of diseases of the central nervous system utilized CSF as the most readily available source of proteins in close proximity to neural tissue.

a) CSF from 100 normal men and women between 20 and 70 years of age have been collected in collaboration with Drs. Berrittini (NIMH), Torrey (St. Elizabeth's Hospital), Burns (Vanderbilt University), Polinsky (NINCHDS), and Sunderland (NIMH). Over 300 CSF proteins per person have been qualitatively assessed, and 8 proteins with polymorphic charge variance identified. Their presumed mendelian inheritance patterns await confirmation in family studies, which are in progress. Otherwise, a remarkably consistent normal CSF pattern has been discovered. In additon to the 26 CSF proteins previously identified by our laboratory, the immunoglobin kappa and lambda light chains have been identified. Quantitative assessment of 68 proteins per individual has revealed no differences between males and females, but levels of 8 proteins were found to change with age: six (including actin) increased and 2 decreased with age. Work is in progress to determine whether these changes are independent of serum proteins (from the same patients).

b) In collaboration with Drs. Asher and Gajduseck (NINCDS) we have identified two abnormal proteins (MW 29kd/pI 5.1 and MW 26Kd/pI 5.2) in CSF from all 21 patients with the infectious dementia known as Creutzfeldt-Jakob disease (CJD). These proteins are not present in CSF from 100 normal persons. CSF from patients with 18 other neurological diseases was studied, and only 50% of patients with Herpes simplex encephalitis (HSE) were found to have the same proteins. HSE is clinically quite distinct from CJD. The presence or absence of these two abnormal CSF proteins was then assessed in a double-blind study of CSF from patients with dementia. All cases of CJD were positively identified in this study, while, no such abnormality was found, in CSF from the other dementias (Alzheimer's disease, Huntington's disease, and parkinsonism dementia of Guam). Electroblots of these two abnormal proteins were probed with polyclonal antiserum to Herpes simplex virus and monoclonal antibodies against immunoglobulin light chains with negative results. Present efforts include the purification of sufficient quantities of the abnormal proteins to enable the determination of the amino acid sequence of at least a portion of these molecules. Experiments can then be pursued to determine their molecular origin. Human samples for this project continue to be obtained in collaboration with Drs Gajduseck, Asher, and Brown (NINCDS).

c) In collaboration with Dr. Torrey (St. Elizabeth's Hospital, Washington, DC) we have studied CSF proteins from patients with schizophrenia, where as yet there is no basic knowledge of underlying biochemical abnormality. 54 patients with schizophrenia were diagnosed by using the DSM III criteria and CSF was obtained. Over 300 CSF proteins per patient were assessed, and 2 abnormal proteins (both of MW 4kd/pI 5.7 and 5.9) that were absent from the CSF from 100 normal persons were found in 17 out of 54 schizophrenic patients. These two proteins were also found in populations with HSE (90%), CJD (67%), Parkinson's disease (12%), multiple sclerosis (13%) and the single patient studied with the Guillain Barre' Syndrome, but they were absent from CSF from patients with 11 other neurological and psychiatric diseases. Immunoprobing has shown that these abnormal proteins are neither Herpes simplex virus-related or antibody proteins. Further research requires purification of these proteins.

In addition to qualitative protein changes, computer assisted quantitation of 68 proteins per individual has shown that 2 CSF proteins were increased by 22% and 27% in the schizophrenic population compared to the normal population, while four proteins were decreased by 29%, 46%, 20%, and 37% ($p<0.005$).

d) In collaboration with Dr. McFarlin (NINCDS) in the previous year, in studies of CSF proteins from patients with multiple sclerosis (MS), we identified qualitative changes of kappa and lambda light chain immunoglobulins and quantitative changes of 25 non-immunoglobulin proteins. This initial study has been pursued by examining the potential use of these newly described abnormalities for better discrimination of multiple sclerosis in MS patients compared to other patients with neurological/psychiatric diseases. In collaboration with Dr. Campbell (DCRT) we have employed discriminant function analysis between multiple proteins in each diagnostic category of MS, other inflammatory or noninflammatory neurological diseases, and normal volunteers. Using this data, we have developed algorithms to distinguish MS from all other diseases with sensitivity and specificity up to 100% in a single population of each diagnostic subpopulation. We are now testing the ability of these algorithms to correctly diagnose patients from a totally new population database.

This work has been further pursued by a study of a large mixed monozygotic and digyzotic twin population with varying concordance/discordance for multiple sclerosis. This study had two aims. One of determining the degree of homology of the abnormal protein changes in affected twins, as compared to any such homology in unrelated individuals, as a measure of genetic control of these characteristic changes. (A population of twins separated at birth would more purely address the genetic question, but such a population is not yet available.) Preliminary results suggest a striking increase in homology of these abnormal proteins in the related as compared to the unrelated individuals. This work is still in progress. The second aim of this study was to compare the ability to detect protein abnormality with greater resolution than that obtained by one dimensional electrophoresis in patients with both established MS and with the recently described subclinical disease. The preliminary results show a high correlation of diagnostic sensitivity with the most sensitive of prior CSF

studies, NMR brain studies and clinical diagnosis, and gave clear evidence of abnormality in clinically normal people who later progressed to develop MS.

e) In collaboration with Drs. Burns and Ebert (Vanderbilt University) we have been studying CSF proteins in Parkinson's disease, MPTP-induced parkinsonism and essential tremor. Last year we observed an abnormal protein MW 25kd, pI 6 in 75% of patients with idiopathic Parkinson's disease. Study of 6 human patients with MPTP-induced parkinsonism showed that three out of the six patients have the same abnormal protein. These three were patients with acute (less than one year) onset of disease. In the remaining three the disease was of eight or nine years duration. The implication of this similar protein appearance in acute toxin-induced disease, where there is highly specific damage to the dopaminergic nigro-striatal pathway, is that the abnormal protein probably arises from damage to this specific cell group. Purification of the protein is in progress, in order to identify its origin by future histochemical and gene-probing studies.

Quantitation of 68 proteins per individual among patients with Parkinson's disease (PD), essential tremor and normal patients revealed 11 proteins altered ($P<.005$) in PD compared to normal persons. Two of these 11 proteins were also altered in pure essential tremor, a disease with very limited molecular abnormality yet identified. Studies on the somewhat similar diseases of the Shy-Drager syndrome and idiopathic orthostatic hypotension in collaboration with Dr. Polinsky (NINCDS) are in progress.

f) In collaboration with Drs. Berrettini, Nurnberger, and Gershon (NIMH), we studied the CSF proteins in manic-depressive disorder (in the well state), with 10 patients both on and off lithium therapy. We found no change as compared to normal persons, and no affect of the lithium therapy. This is of interest in the light of positive changes in all other sixteen diseases studied so far.

g) Other CSF studies of human diseases of the nervous system that are in progress, but without sufficient data on which to report, include: Alzheimer's disease with Dr. Sunderland (NIMH), Wernicke's encephalopathy with Dr. Martin (NIAAA), Huntington's disease with Dr. Manyam (Univ So Illinois), amyotrophic lateral sclerosis with Dr. Dalakos (NINCDS).

h) In collaboration with Dr. Stanley (University of Detroit) we have initiated a pilot study of proteins obtained from postmortem samples. The strategy involves collection (by Dr. Stanley) of CSF, brain and blood, shortly after death, from individuals who have no history of disease involving the CNS. Such samples of human tissue may have advantages over samples from premortem clincal practice. Dr. Stanley has already shown that many of the normal CSF small peptide gradients are maintained in postmortem specimens. Should this prove true for the larger proteins of our studies (14-200kd), this will supply a useful source of proteins from both normal and disease states.

B. In collaboration with Drs. Price and Cork (Johns Hopkins University) we are trying to define the biochemical basis of the gene defect in the

autosomal dominant canine model of amyotrophic lateral sclerosis. Our strategy is to select ventral horn spinal cord tissue from homozygote, heterozygote and unaffected dogs in the disease model that has been developed at Johns Hopkins University. We are studying protein changes by initial 2DE survey and by probing with neurofilament antibodies, which are known to be abnormally accumulated in ventral horn disease. Preliminary studies have shown 55kd proteins to be present in affected but not in normal dogs. Our objective is to identify protein changes that correlate with gene dosage as a means of identifying the gene defect. Subsequent studies of human amyotrophic lateral sclerosis will be considered once a protein/gene marker has been obtained.

C. In collaboration with Dr. I. Hay we are studying the affect of viruses (predominantly Herpes simplex virus) on varied cultured human cell lines, including skin fibroblasts and neuroblastoma. This work is aimed at identifying the virus-induced protein changes (over 230 occur in Herpes simplex virus-infected skin fibroblasts) that correspond to protein changes in diseases of the nervous system with a suspected viral etiology.

D. In collaboration with Drs. Voisin and Klein (LDN, NICH) we have identified a protein of MW 37,pI 6 that is induced ten-fold by norepinephrine in normal bovine pineal glands, both in-vivo and in-vitro. This induction occurs extremely quickly (within 1-2 hours of stimulation). The basic interaction, pharmacology and biochemistry of this receptor-mediated protein production is being pursued.

Significance:

The application of two dimensional electrophoresis for the study of inherited protein polymorphisms and their potential linkage to disease gene loci, and the physiological and pathological approach to CSF/spinal cord/viral proteins is yielding considerable new basic biochemical information in many poorly defined human disease processes. For instance, the abnormal CSF proteins in CJD appear to have diagnostic potential for distinguishing an infectious form of dementia from the more common dementias including Alzheimer's disease. Because of accidental transmission of CJD, this is likely to be of clinical value. The potential for further study of the many abnormalities in the diseases described has obvious relevance to the NIMH. Furthermore, the technological advances in protein detection and analysis developed in this section have wide applicability for fellow scientists both within the NIMH and in the wider scientific community.

Identification of polymorphic proteins, as demonstrated in two dimensional electrophoretic studies, will compliment the use of restriction length DNA polymorphisms in the generation of a set of linkage markers for the human genome. If a sufficient number of linkage markers were available, it would be possible to establish genetic linkage with genetic disease loci. The human genome contains 3,300 recombinational units or "centimorgans" (a centimorgan represents a recombinational distance such

that any gene within one centimorgan will be linked 99% of the time. It physically represents about one million base pairs of DNA). If the medical community had a collection of 165 evenly spaced linkage markers, each 20 centimorgans from the next, than any disease locus would be within 10 centimorgans from one of the linkage markers and would be linked to such a marker 90% of the time. A collection of such linkage markers would be invaluable both as a diagnostic tool and as a means of finding disease loci so that the molecular basis of each genetic disease could be determined, hopefully permitting rational therapies. A collection of 165 evenly spaced loci does not yet exist, however, it is estimated that 200 randomly spaced linkage markers would provide a collection of markers speaced no more than 20 centimorgans apart for 80% of the human genome. The identification of protein polymorphisms currently offer at least 27 such linkage markers for use in studies of human genetic disease.

Proposed course:

Identification and scoring of polymorphic and disease associated proteins has been performed by a combination of computerized microdensitometry and manual protein identification. We hope to develop and employ fully automated methods in the analysis of two dimensional electrophoretograms. Such automated analysis will permit genetic disease linkage studies utilizing identified polymorphic proteins as markers in larger clinical studies. Quantitative computerized analysis of electrophoretograms should also permit the identification of additional polymorphic markers, including uncharged variants and quantitatively polymorphic proteins. Almost 2/3 of all amino acid substitutions cause no change in the charge of a protein, but may alter its stability. The ability to quantitatively scan a large number of proteins offers the posibility of scoring these amino acid substitutions, thereby expanding our library of genetic linkage markers.

We also propose to maintain and continue development of sensitive methodology for protein detection and analysis of complex protein mixtures, and to pursue disease-associated protein changes in the disorders of the central nervous system under scrutiny.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01836-07 NS

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptors in the Central Nervous System: Biochemistry to Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

High affinity and stereospecific receptors for benzodiazepines are present in the mammalian central nervous system. It is currently believed that the interaction of benzodiazepines with their receptors initiates a series of neuronal events resulting in an enhancement of GABA-mediated choloride permeability. The latter results behaviorally in the major pharmacological actions of benzodiazepines, namely their anxiolytic, anticonvulsant, hypnotic, and muscle relaxant actions. In addition to benzodiazepines, a variety of sedative/hypnotic agents of the minor tranquilizer class (e.g., the barbiturates) appear to interact with one or more components of the benzodiazepine/GABA receptor complex, and thus the latter has been proposed as a common site of minor tranquilizer action. Several aspects of the benzodiazepine/GABA receptor complex are currently being studied, including purification of the receptor, charaterization of multiple binding sites on the receptor complex which recognizes agonist, antagonists or inverse agonists. The development of anti-iodiotypic antibodies to the various binding site domains on the complex studies on the behavioral and biochemical effects of novel (non-benzodiazepine) anxiolytics as well as "anxiogenic" inverse agonists, and the identification of a novel benzodiazepine receptor in the CNS and peripheral tissues for 4-chlorodiazepam (Ro5-4864), the so-called peripheral benzodiazepine receptor ligand. Recent work has also focused on using an in vitro system for measuring GABA receptor-effector coupling in a subcellular preparation from rat brain (the synaptoneurosome). This technique has greatly facilitated studies on barbiturate and GABA receptor-mediated chloride flux and has resulted in the first reliable method for studying the function of the GABA receptor in vitro.

Other Professional Personnel:

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G. T. Bolger	Visiting Fellow	LBC, NIADDK
C. B. Pert	Pharmacologist	NS, NIMH
R. J. Weber	Staff Fellow	NS, NIMH

Project Description:

Objectives:

1. To completely characterize the interaction of anxiogenic and anxiolytic compounds with the benzodiazepine/GABA receptor complex at the molecular/cellular and behavioral levels.

2. To understand the mechanism(s) responsible for the stress-induced "sensitization" of this receptor complex and to unravel whether changes in the ionophore underlie the development of tolerance to sedative/hypnotic/anxiolytic drugs.

3. To use the GABA receptor/ionophore complex as a model of other neurotransmitter - gated ion channels.

4. To explore basic neurochemical mechanisms of anxiety, fear and stress as they relate to the many clinical and medical problems associated with stress.

Methods Employed:

(See: 1984 Annual Report, pp. 699-706, Project Number Z01 MH 01836-06 NS, Receptors in the Central Nervous System: Biochemistry to Behavior.)

Major Findings:

Agents that perturb one or more of the components of the benzodiazepine-GABA receptor chloride ionophore complex (e.g., benzodiazepines, GABA and GABAmimetics like muscimol, and barbiturates) have been shown to increase chloride conductance in both electrophysiological preparations and intact cells. Nonetheless, attempts to develop a quantitative measurement of chloride flux in a "cell-free" preparation (in order to explore the functional relationship of the supramolecular complex) have proven unsuccessful. Such studies have employed modifications of the "classical" synaptosome preparation of Gray and Whittaker which consists of resealed presynaptic vesicles with attached postsynaptic membranes. We have employed a "filtered synaptoneurosome" preparation (developed by Hollingsworth, Creveling and Daly) for the study of ^{36}Cl-flux. The filtered synaptoneurosome preparation was employed because it

has been shown to contain both presynaptic nerve endings and attached postsynaptic densities. We have shown that pentobarbital causes a concentration dependent increase in the efflux and uptake of ^{36}Cl-efflux from preloaded synaptoseurosome which is reversed by the chloride ionophore antagonist, pictrotoxin. A good correlation ($r = 0.90$, $p < 0.01$) was obtained between the potencies of a series of barbiturates in increasing ^{36}Cl-efflux from preloaded synaptoneurosomes and their anesthetic potencies in mice. Furthermore, the potencies of these barbiturates to stimulate ^{36}Cl-efflux was correlated ($r = 0.77$, $p < 0.05$) with their potencies to enhance [^{3}H]diazepam binding in cerebral cortical membranes. The GABAmimetic muscimol also increased ^{36}Cl-efflux, and this effect was reversed by the GABA antagonist bicuculline. The efficacy of pentobarbital in stimulating ^{36}Cl-efflux in a number of brain areas was highly correlated with the relative densities of [^{35}S]t-butylbicyclophosphorothionate (TBPS) binding sites in these areas ($r = 0.96$, $p = 0.01$), which suggests the chloride efflux measured in this system may reflect the $GABA_A$ receptor-chloride ionophore linked to benzodiazepine receptors. Thus, the synaptoneurosome appears to be an excellent model for studying the effects of pharmacologic, biochemical, and behavioral manipulation on chloride flux to a population of channels that are linked to both GABA and benzodiazepine receptors.

Despite both direct and correlative evidence that suggests benzodiazepine receptors mediate the antianxiety (anxiolytic) actions of the benzodiazepines (e.g., diazepam, chlordiazepoxide) and related compounds, the physiological function(s) of these sites is unclear. Nonetheless, several lines of evidence suggest these receptors may play a role in the endogenous control of anxiety. For example, studies from this and other laboratories have shown that certain C-3 substituted β-carbolines elicit somatic, endocrine, and behavioral effects reminiscent of stress of anxiety in both rodents and primates, including man. Furthermore, Guidotti, et al. (1983) have isolated a protein (molecular weight ∿11 kdalton) that binds to benzodiazepine receptors with a moderate affinity (IC_{50} ∿4 μM), and is reported to have "anxiogenic" properties. Nonetheless, studies attempting to demonstrate alterations in benzodiazepine receptors as a result of "stress" or "anxiety" in experimental animals have been disappointing in that the changes in these receptors have been small and not unidirectional. We have recently demonstrated that several stressors (forced ambient temperature swim, brief immersion in ice water, and food deprivation for the first three hours of the dark cycle) elicit a rapid and robust change in [^{3}H]benzodiazepine binding that is observed only in the presence of Eccles' permeable anions (e.g., chloride, iodide and bromide ions). No differences in either basal or GABA-enhanced [^{3}H]benzodiazepine binding was observed between stressed and control animals. These differences are manifest as an increase in the apparent affinity of [^{3}H]flunitrazepam, with no significant differences in the maximum number of binding sites (B_{max}) between the groups. Both an increase in the maximum enhancement of [^{3}H]flunitrazepam binding in response to optimum concentrations of halide ions (E_{max}) and an increased sensitivity to halide ions (reduced EC_{50}) were observed in response to stress. These results suggest that acute exposure to stress affects either the coupling between the chloride ionophore and benzodiazepine receptor or the chloride ionophore iteself rather than the benzodiazepine receptor per se. These data suggest that GABA receptor-mediated Cl conductance would be more efficient in "stressed" animals and, in fact, recent studies using the synaptoneurosome preparation have confirmed this

rapid "sensitization" of GABA receptors following "stress". The mechanism(s) responsible for this post-translational modification of the GABA receptor complex are currently being investigated.

Many membrane associated receptors have been shown to be sensitive to alterations in their lipid milieu. Changes in membrane lipids induced by activation of phospholipase A_2 (PLA_2) (an endogenous constituent of membranes) has been proposed as a physiologic mechanism for regulating receptor function. We have shown a differential sensitivity of "peripheral" and "central" benzodiazepine receptors to this enzyme. Furthermore, the components of the benzodiazepine-GABA receptor chloride ionophore complex (supramolecular complex) are differentially sensitive to this enzyme. Phospholipase A_2 slightly increased the apparent affinity of the central benzodiazepine receptor ligands [^{3}H]flunitrazepam and [^{3}H]3-carboethyoxy-β-carboline, with no concomittant change in the B_{max} of these ligands. In contrast, GABA enhanced [^{3}H]flunitrazepam was unaffected by PLA_2. Both pyrazolopyridine and barbiturate enhanced [^{3}H]flunitrazepam binding were, however, reduced by very low (0.002 U/ml) concentrations of PLA_2. Since both pyrazolopyridines and barbiturates bind to sites at or near the chloride ionophore, we examined the effects of PLA_2 on the specific chloride ionophore ligand [^{35}S]t-butylbicyclophosporothionate (TBPS). It was found that PLA_2 inhibited [^{35}S]TBPS binding at the same concentrations needed to disrupt barbiturate and pyrazolopyridine enhanced [^{3}H]flunitrazepam binding. The inhibition of [^{35}S]TBPS binding by PLA_2 was manifest as a reduction in the B_{max} of this ligand with no change in the apparent affinity. PLA_2 was also found to reduce the apparent affinity of [^{3}H]Ro5-4864 to "peripheral" benzodiazepine receptors (PBR) without affecting the B_{max} of this compound. The magnitude of reduction in the apparent affinity of [^{3}H]Ro5-4864 was independent of this tissue source (i.e. the same reduction in apparent affinity was found in heart, kidney and brain membranes). In contrast, PLA_2 treatment of heart membranes increased the binding of [^{3}H]PK-11195, an isoquinoline derivative that binds to PBR and has been postulated to be an "antagonist" (while Ro5-4864 is an agonist) at these sites. These findings demonstrate that both "central" and "peripheral" benzodiazepine receptors are differentially sensitive to alterations in their lipid microenvironments; and these differences are ligand-specific.

Although benzodiazepines have been shown to augment the electrophysiological actions of GABA (the prinicpal inhibitory neurotransmitter of mammalian brain), there is still controversy over the relationship between this effect and pharmacologic actions of the benzodiazepines. For example, it has been difficult to consistently mimic the action of benzodiazepines with GABA or pharmacologic manipulation of GABAergic pathways. Direct application of the GABAmimetic muscimol into the lateral septum which results in an increased anticonflict action in rats at does of 0.1-5 ng. Higher doses of muscimol reduce anticonflict behavior and produce sedation. Nonetheless, the maximum dose of muscimol (5 ng) that elicits an anticonflict effect did not significantly affect non-punished responding. These observations demonstrate that under appropriate experimental conditions, a consistent, anticonflict action of muscimol may be observed, suggesting that GABAergic pathways may play a role in the anticonflict actions of the benzodiazepines.

Since β-carbolines can mimic the somatic, endocrine and affective symptoms of "anxiety" or "fear" in both rodents and primates, we have explored the possiblity that chemically induced "anxiety" may affect the immune system. Twenty-four hours after a single dose of the β-carboline FG 7142 (N-methyl-β-carboline-3-carboxamide) to rats, spleens were removed, the spleen cells cultured, and subsequently challenged with the mitogens concanavalin A (Con A) and phytohemagglutin A (PHA). The response of spleen cells derived from rats injected with FG 7142 to these mitogens was reduced by >80% compared to vehicle injected animals, suggesting the immune system of these animals may be compromised. Pretreatment of animals with the benzodiazepine receptor antagonist Ro15-1788 resulted in a partial to complete reversal of this apparent immunosuppression.

We have previously shown that the prototype benzodiazepine ligand for PBR, Ro5-4864 (4'-chlorodiazepam) is a potent convulsant. The inability of either Ro15-1788 or PK-11195 to antagonize this effect suggets the convulsant actions of Ro5-4864 are not related to direct occupation of either peripheral or central benzodiazepine receptors. However, these studies did not rule out the possibility that another component of the supramolecular complex is involved in the convulsant action of Ro5-4864. Examinations of a series of compounds that are structurally related to Ro5-4864 revealed no correlation between the convulstant potencies of these compounds and their affinities for PBR. In contrast, a good correlation ($r = 0.93$, $p < 0.01$) was obtained between the affinities of these compounds for the chloride channel coupled to central benzodiazepine receptor (as measured by displacement of [^{35}S]TBPS) and their convulstant potencies. Thus, the convulsant action of Ro5-4864 and related compounds appears to be related to a picrotoxinin-like action and may not be directly related to the PBR.

Acidified methanol or trichloroacetic acid extraction of peripheral tissues and brain followed by ultrafiltration and/or gel chromatography has revealed the presence of both high (Mr 10 kdalton) and low (Mr 0.5 kdalton) molecular weight substances which inhibit the binding of [^{3}H]Ro5-4864 to PBR but does not affect the binding of [^{3}H]Ro15-1788 to central benzodiazepine receptors. The high degree of hydrophobicity of the larger molecular weight substance resulted in a more efficient and rapid preliminary isolation on Sep-Pak ODS columns followed by purification to near homogeneity using multiple reverse phase HPLC followed by gel filtration HPLC. This high molecular weight fraction, which we have termed "antralin", reduces the specific (but not nonspecific) binding of [^{3}H]Ro5-4864 by reducing the apparent affinity of [^{3}H]Ro5-4864 without altering the maximum number of PBR. Antralin is unevenly distributed among various tissues and is destroyed by both heat treatment and pronase. Antralin also inhibits the binding of [^{3}H]dihydropyridine calcium channel antagonists (e.g., nifedipine) with equal potency. Furthermore, the inhibitory activity of 10-15 kdalton protein is enhanced by the presence of physiological concentrations of calcium ions. Chromatographic separation of antralin has conclusively shown that the same molecule affects both PBR and voltage sensitive calcium channels. The widespread distribution of PBR coupled with the established physiological importance of voltage sensitive calcium channels suggests that antralin may represent the first of a new family of regulatory proteins.

Inbred mice have been used in an attempt to define the function of "peripheral-type" benzodiazepine receptors (PBR) in both peripheral tissues and central nervous system using a combination of behavioral, physiological, neurochemical, and genetic techniques. Differences in the susceptibility of different inbred mouse strains to Ro5-4864 were studied to better define the neurochemical basis for such inherent differences. Screening of eight related inbred strains of mice resulted in three strains, DBA/2, BALB/cBy and SWR with significant dose dependent differences in their susceptibilities to the convulsant actions of Ro5-4864. Pharmacokinetic factors were eliminated as a contributing factor since no differences were found in the brain concentrations of Ro5-4864 at the time of convulsions in these strains. The hyporesponsive strains (SWR and BALB/cBy) were found to have a reduced number of PBR in the forebrain compared to the sensitive (DBA/2) strain. A similar hyporesponsiveness to picrotoxinin and related convulsants were found in these strains, which is consistent with other findings from this laboratory that suggest part of the convulsant actions of Ro5-4864 may be attributed to an action at the chloride ionophore. However, no differences in the sensitivity to two unrelated convulsants, bicuculline and strychnine, were observed. Furthermore, no differences in the binding of [^{35}S]TBPS were observed among these strains. Synaptoneurosomes prepared from a hyporesponsive an sensitive strain of mouse revealed that picrotoxinin-induced blockade of ^{36}Cl-efflux was greater in the sensitive strain. Conversely, pentobarbital was more potent in protecting this strain *in vivo* from picrotoxinin induced seizures. This study demonstrates for the first time an inherited difference in the sensitivity of the benzodiazepine-GABA receptor chloride ionophore complex.

Significance to Biomedical Research and Program of the Institute:

Many of the pharmacologic agents described in these studies are widely used or abused substances. Understanding the mechanisms by which these compounds exert their pharmacologic actions are of fundamental importance for a better understanding of epilepsy, anxiety-neuroses, sleep disorders, and depression. These studies may also clarify the role of ion transport in the mechanisms of action of these agents, and could result in new insights of how aberrations in ion transport could lead to pathological states. These studies can also provide valuable information leading to the development of more efficacious and safer therapeutic agents.

Proposed Course:

The quantitation of ^{36}Cl-efflux and uptake in synaptoneurosomes appears to be a relevant measure of the effector system in the benzodiazepine-GABA receptor chloride ionophore complex. This system will be used in conjunction with other studies from this laboratory on stress of anxiety related changes in the "supramolecular complex". Initial studies will focus on the effects of acute stress and inescapable shock on changes in chloride efflux. Since very low levels of phospholipase have been shown to affect [^{35}S]TBPS binding, which is a marker for the functional state of the chloride ionophore, studies will be performed to determine whether ^{36}Cl-efflux is similarly affected. Exploratory studies will also be done on measurement of ^{36}Cl-efflux. The demonstrations of rapid and robust stress induced changes in the "supramolecular complex" will

result in future studies to determine how the chloride ionophore itself can be rapidly modulated by "stress". Pharmacologic studies will be performed to determine whether the effects of acute stress can be muted or blocked with anxiolytic drugs, and whether the effects of stress may be mimicked by administration of anxiogenic agents. The contribution of the hypothalamic pituitary-adrenal axis on these rapid, stress induced changes will also be examined. Anxiogenic β-carbolines have been shown to compromise the immune response. Further studies will determine the duration of this immunosuppression, and whether anxiolytic agents can reverse this effect. The effects of chronic treatment with anxiogenic agents on immune response will also be explored, as will the contribution of the pituitary-adrenal axis.

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3. Skolnick, P., Paul, S.M. and Weissman, B.A. Preclinical actions of buspirone. Pharmacotherapy 4: 308-314, 1984.

4. Bolger, G., Mezey, E., Cott, J., Weissman, B.A., Paul, S.M. and Skolnick, P. Differential regulation of "central" and "peripheral" benzodiazepine binding sites in rat olfactory bulb. Eur. J. Pharmacol. 105: 142-148, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02186-03 NS

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Recognition Sites for Stimulants and Antidepressants: Relationship to Pharmacological Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	S. M. Paul	Chief	NS, NIMH
Others:	I. Angel	Guest Researcher	NS, NIMH
	P. Skolnick	Pharmacologist	LBC, NIADDK
	M. E. Goldman	Staff Fellow	NS, NIMH
	R. Labarca	Guest Researcher	NS, NIMH
	J. N. Crawley	Res. Biologist	NS, NIMH
	R. D. Schwartz	Pharmacologist	NS, NIMH

COOPERATING UNITS (if any)
Laboratory of Bioorganic Chemistry, NIADDK; Section on Molecular Pharmacology, NS, NIMH; Section on Clinical Studies; NS, NIMH.

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Section on Preclinical Studies

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recognition sites for a variety of psychotherapeutic drugs have been identified in the central nervous system. Several of these binding sites, including those for benzodiazepines, opiates, and various neuroleptics have subsequently been shown to be true pharmacological receptors in that the binding of drug to its respective recognition site is a necessary (and many times sufficient) requirement for drug action. Over the past several years we have attempted to identify recognition sites for other common psychotropic drugs incuding tricyclic antidepressants and the psychomotor stimulants, amphetamine and methylphenidate. In each case saturable, and stereospecific binding sites have been delineated; and for amphetamine and methylphenidate relatively good correlations have been observed between the affinities of a series of analogues in vitro and at least some of the pharmacological properties of these agents. Tricyclic antidepressants including imipramine and desipramine, also bind to distinct recognition sites that are functionally and structurally associated with the presynaptic uptake sites for serotonin and norepinephrine respectively. Thus, radiolabeled antidepressants have been useful probes in studying the mechanisms of neurotransmitter uptake in both central and peripheral tissues, and under a variety of clinical conditions. Recent work has shown that the [^{3}H] (+)-amphetamine binding site in hypothalamic membranes is sensitive to circulating levels of blood glucose. Hypoglycemia decreases, and hyperglycemia increases, the number of [^{3}H] (+)-amphetamine binding sites in hypothalamic membranes respectively. Furthermore, these changes seemed to be coupled to the activity of (Na^+ K^+) (ATPase; and there is a good correlation between the changes in [^{3}H] (+)-amphetamine and [^{3}H]-ouabain binding both in vivo and in vitro. More recent studies have shown that [^{3}H]-mazindol a chemically unrelated anorectic/psychostimulant also can be used to label the [^{3}H] (+)-amphetamine recognition site and that there is a good correlation between the inhibition of [^{3}H]-mazindol binding by a series of phenylethylamines and their anorectic potencies in rats.

Other Professional Personnel:

K. C. Rice Staff Fellow LC, NIADDK

Project Description:

Objectives:

1. To elucidate the mechanisms of action of important psychotropic drugs such as the psychomotor stimulants and antidepressants.

2. To identify novel receptor-effector systems in brain that are operative in chemical neurotransmission.

Methods Employed:

(See: 1984 Annual Report, pp. 707-712, Project Number Z01 MH 02816-02 NS, Brain Recognition Sites for Stimulants and Antidepressants: Relationship to Pharmacological Activity.)

Major Findings:

High affinity, stereospecific binding sites for [^{3}H] (+)-amphetamine have been previously described in rodent brain. The highest density of these sites are found in the synaptosomal fraction of brain stem and hypothalamus. A striking correlation ($r = 0.97$; $p < .01$) has been demonstrated between the ability of a series of amphetamine derivates in displacing [^{3}H] (+)-amphetamine from these sites and their potencies as anorectic agents. Recently, a similar population of low affinity (Kd = 8-10 μM) high capacity binding sites for [^{3}H]-mazindol have been identified in brain which are similar if not identical to the [^{3}H] (+)-amphetamine binding site. These observations suggest that the [^{3}H]-mazindol amphetamine binding site may be involved in the appetite suppressant actions of chemically unrelated anorectic agents. [^{3}H] (+)-Amphetamine binding has also been studied in genetically obese mouse strain. In these animals, the density of hypothalamic [^{3}H] (+)-amphetamine binding sites is greater than in lean litter mate controls. Furthermore, food deprivation of rats (24-72 hours) results in a dramatic (35-50%) reduction in the density of hypothalamic [^{3}H] (+)-amphetamine binding sites. Refeeding food-deprived animals for a four hour period (or allowing access to a 10% glucose solution) results in a return of [^{3}H] (+)-amphetamine binding site density to control values. These data suggest that the [^{3}H] (+)-amphetamine binding sites may be intimately involved in the regulation of feeding behavior of animals. In more recent experiments the changes in [^{3}H]-mazindol/amphetamine binding during food deprivation and refeeding have been localized to discrete hypothalamic and brainstem nuclei (paraventricular nucleus and nucleus tractus solitarius).

Since [^{3}H] (+)-amphetamine/mazindol binding in hypothalamus is decreased following 24 hours of food deprivation (30% reduction in B_{max}), and the site density restored to control levels if the animals are permitted to refeed for four hours we have examined the factors responsible for this rapid modulation in site number. Recent studies have now demonstrated that intraperitoneal

injection of 2-deoxy-D-glucose elicits a significant increase in [^{3}H] (+)-amphetamine binding in the hypothalamus and brainstem. This treatment did not alter [^{3}H] (+)-amphetamine binding in other brain regions. Injection of L-glucose failed to elicit an increase in [^{3}H] (+)-amphetamine binding. Further, injection of 2-deoxy-D-glucose elicited a similar increase in binding site density and these changes were again confined to the PVN and NTS. Interestingly, if animals are permitted access to food during the four hour interval following injection of the 2-deoxy-D-glucose, the increase in [^{3}H] (+)-amphetamine binding is not observed. These observations suggest that [^{3}H] (+)-amphetamine/mazindol binding sites in the hypothalamus are coupled to glucose utilization. The high correlation previously reported between the ability of a number of phenethylamines to inhibit [^{3}H] (+)-amphetamine binding and their potencies as anorectics may thus link the anorectic actions of phenethylamines with their ability to effect glucose-responsive neurons in the hypothalamus.

In related experiments the regulation of [^{3}H] (+)-amphetamine binding in hypothalamic tissue slices in vitro have confirmed that glucose plays a major role in determining the density of [^{3}H] (+)-amphetamine binding sites. The effects of neurotransmitters on the [^{3}H] (+)-amphetamine/mazindol binding was suggested by examining the effects of neurotoxins. Six-hydroxydopamine lesions increased [^{3}H] (+)-amphetamine binding in striatal membranes but had no effect in the hypothalamus. In contrast, 5,7-dihydroxytryptamine lesions resulted in a significant increase in [^{3}H]-amphetamine/mazindol binding in hypothalamic membranes suggesting that serotonin may modulate these sites in hypothalamus. Recent preliminary experiments do, in fact, demonstrate that serotonin will increase [^{3}H] (+)-amphetamine binding when added to hypothalamic slices in vitro.

Previous studies in our laboratory have demonstrated the presence of high affinity, stereospecific binding sites for [^{3}H] (±) threo-methylphenidate in the striatum and brainstem of the rat. Subsequent studies have demonstrated that the binding of [^{3}H]-methylphenidate is localized to synaptosomes, and that the binding is dependent on the presence of sodium. Intraventricular administration of 6-hydroxydopamine or medial forebrain bundle lesions results in a significant loss of [^{3}H]-methylphenidate binding in striatum which is highly correlated with a loss in the capacity of this tissue to take up [^{3}H]-dopamine. Structure-activity studies suggest that this site is associated with a dopamine transport system since a high correlation ($r = 0.88$, $p < .001$) was found between the potencies of a series of compounds to inhibit [^{3}H]-dopamine uptake and inhibit [^{3}H]-methylphenidate binding. These findings suggest the methylphenidate binding site may be part of a dopamine "transporter", analogous to findings demonstrating that [^{3}H]-imipramine and [^{3}H]-desipramine label components of the serotonin and norepinepherine "transporters", respectively. Further, pilot studies in human autopsy material suggest a distribution of site density similar to that found in rat, the highest site densities observed in striatum, and lowest in cerebellum. In a related series of experiments several diphenyl-substituted piperazines (GBR-12935, GBR-12921) have been tested for their selectivity in inhibiting dopamine uptake. The marked specificity of these compounds in inhibiting dopamine uptake has prompted the radioactive labeling of GBR-12935. [^{3}H]-GBR-12935 appears to be a "super high affinity" ligand for the

dopamine uptake site and may be useful for in vivo imaging of dopamine-containing neurons. Previous work in our laboratory have demonstrated that [^{3}H]-imipramine labels the "serotonin transporter" (recognition site + transport protein) in brain and platelets of both human rat. Since the number of these sites is reduced in platelets of depressed patients, this parameter could be an important marker for depressive illness.

The hydrolysis of phosphotidylinositol (PI) or the polyphosphoinositides is now recognized as a "second messenger" system mediating signal transduction for a variety of hormones and neurotransmitters. PI "turnover" is dramatically altered by lithium, since the latter is a rather potent inhibitor of inositol phosphatase and thus the recycling of inositol. We, and others, have capitalized on the use of lithium to amplify the effects of various neurotransmitter agonists in causing an accumulation of inositol-1-phosphate in vitro. Thus, using brain slice preparations we have characterized the effects of various neurotransmitters in stimulating PI hydrolysis and have used this system for studying the regulation of PI-coupled receptors. To date, we have examined the effects of various chemical and surgical lesions on neurotransmitter-mediated PI turnover in discrete brain regions. In addition, we have observed that activation of protein kinase C by phorbol esters markedly inhibit the neurotransmitter-stimulated turnover of PI; indicating the possible existence of a negative feedback loop.

More recently we have characterized a subcellular preparation from brain that is suitable for studying PI turnover. Using this preparation we have discovered the presence of multiple pools of PI that are regulated by manganese and CDP:diacyclglycerol. At least one pool of PI is sensitive to agonist-induced hydrolysis and the other is not. The possible physiological significance of these pools will be investigated further.

Significance to Biomedical Research and Program of the Institute:

All of the drugs under investigation have important psychotropic and behavioral actions and are either of therapeutic benefit or reliably mimic various behavioral states. Thus an understanding of their mechanisms of action should be of value to understanding the behavioral and psychopathological states responsive to treatment with these agents.

Proposed Course:

Studies will continue on the various recognition sites described above to more fully elucidate their pharmacological as well as physiological significance. A major emphasis will be placed on defining the alterations in [^{3}H] (+)-amphetamine/mazindol binding that occur in vivo during various manipulations of "appetite" and "satiety", in order to test the hypothesis that these sites are coupled to a physiological mechanism regulating food intake (particularly carbohydrate intake) in animals. The relationship between the [^{3}H] (+)-amphetamine binding and the neuronal form of NA^+ K^+ ATPase will also be investigated since the latter is one of the most important "utilizers" of energy derived from glucose. Emphasis will be placed on whether these binding sites label some novel postsynaptic effector system and whether conventional neurotransmitters such as dopamine and sertonin alter these sites in vitro.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00112-08 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endorphin Research in Mental Illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Pickar	Chief, Section on Clinical Studies	NS, NIMH
Others:	G.A. Roy	Visiting Associate	NS, NIMH
	O.M. Wolkowitz	Medical Staff Fellow	NS, NIMH
	A.F. Breier	Medical Staff Fellow	NS, NIMH
	M. Dubois	Staff Member, Department of Anesthesia Georgetown Medical School	
	T.N. Wise	Chief of Psychiatry, Fairfax Hospital	

COOPERATING UNITS *(if any)*

Laboratory of Psychology and Psychopathology, NIMH; Georgetown Medical School; Fairfax Hospital

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies the role of the endogenous opioid system (EOS) in humans. We have previously completed a dose-response study in normals of high doses of the opiate antagonist, naloxone (up to 4 mg/kg). Significant dose-dependent increases in physiologic (blood pressure and respiratory rate) and hormonal variables (cortisol and growth hormone) were found suggesting progressive EOS blockade with increasing naloxone doses. Normals also experienced dysphoria at high naloxone doses suggesting EOS involvement in the regulation of mood in normals. In a separate double-blind study, high doses of naloxone produced a significant decrease in caloric intake in healthy, normal volunteers supporting hypothesized involvement of the EOS in eating behavior. We have recently added to these data by demonstrating significant naloxone-induced reductions in eating behavior in obese subjects. In previous work, we have studied the relationship between the hypothalamic-pituitary-adrenal axis and the EOS in depressive illness. We have recently observed increased cortisol response to naloxone in both drug-free and medicated schizophrenic patients in comparison to controls. Further work in depressed patients using this paradigm is indicated.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION

The major aim of this project is to study the roles of the endogenous opioid system (EOS) in human behavior and physiology and in psychiatric illness. We have used naloxone administration strategy to study the tonic role of the EOS in humans. We have further studied links between hypothalamic-pituitary-adrenal (HPA) axis and the EOS in depressed patients. Finally, we have continued to investigate the response of endogenous opioids to stress and its relationship with endogenous analgesic mechanisms.

METHODOLOGY

(See: 1984 Annual Report, pp 713-717, Project Number Z01 MH 00112-07 NS, Endorphin Research in Mental Illness)

MAJOR FINDINGS

1. We have observed a significant naloxone-induced (25%) reduction in eating behavior during the course of a 24-hour period in obese subjects. These data are consistent with our other results from studies of normal controls and suggest the possibility that opiate antagonists might have a clinical role in treating patients with enhanced eating behavior.

2. Applying the naloxone challenge strategy to schizophrenic patients we have recently observed enhanced cortisol response to naloxone (0.5 mg/kg) in both drug-free and medicated schizophrenics in comparison to age- and sex-matched controls. These data are consistent with the notion of enhanced endogenous opioid system tone in schizophrenia and their further examination in prospective studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

The endogenous opioid system has been one of the most important scientific discoveries of the last several decades. Over the past years our program has worked towards systematically studying the role of this system in normal human behavior. As part of this ongoing research we have observed further evidence suggesting involvement in the endogenous opioid system and eating behavior in obese subjects, a finding which may have potential treatment significance. Further, we have gained evidence of enhanced cortisol response to naloxone in schizophrenic patients, another piece of evidence suggesting endogenous opioid system involvement in schizophrenia.

PROPOSED COURSE

We intend to pursue naloxone stimulated increases in cortisols in various psychiatric diagnostic groups. We feel that this paradigm is useful in studying endogenous opioid system functioning in these illnesses. We are currently evaluating future studies in obese subjects using administration of naloxone.

PUBLICATIONS

Bullinger, M., Naber, D., Pickar, D., Cohen, R. M., Kalin, N. H., Pert, A., and Bunney, W. E., Jr.: Endocrine effects of the cold pressor test: relationships to subjective pain appraisal and coping. Psychiatry Res., 12: 227-233, 1984.

Cohen, M. R., Cohen, R. M., Pickar, D., and Murphy, D. L.: High dose naloxone in depression. Biol. Psychiatry, 19: 825-832, 1984.

Cohen, M. R., Cohen, R. M., Pickar, D., and Murphy, D. L.: Naloxone reduces food intake in man. Psychosomatic Med., 47: 132-138, 1985.

Cohen, M. R., Pickar, D., and Cohen, R. M.: High-dose naloxone administration in chronic schizophrenia. Biol. Psychiatry, 20: 570-583, 1985.

Cohen, M. R., Pickar, D., Cohen, R. M., Wise, T.N., and Copper, J.N.: Plasma cortisol and β-endorphin immunoreactivity in human obesity. Psychosom. Med., 46: 454-462, 1984.

Naber, D., Bullinger, M., and Pickar, D.: Neuroendocrine, psychological and psychophysiological variables in human stress response. In Pancheri, P., Zichella, L., and Falaschi, P. (Eds.): Endorphins, Neuroregulators and Behaviour in Human Reproduction, Proceedings of the 3rd International Symposium on Psychoneuroendocrinology in Reproduction, Spoleto, Italy, 9-12 July, 1982. Amsterdam, Excerpta Medica, 1984, pp 256-266.

Naber, D. and Pickar, D.: Endorphine und endogene psychosen. Nervenarzt, 55: 378-381, 1984.

Pickar, D., Cohen, M. R., Naber, D., and Post, R. M.: The endogenous opioid system in human behavior. In Pancheri, P., Zichella, L., and Falaschi, P. (Eds.): Endorphins, Neuroregulators and Behaviour in Human Reproduction, Proceedings of the 3rd International Symposium on Psychoneuroendocrinology in Reproduction, Spoleto, Italy, 9-12 July, 1982. Amsterdam, Excerpta Medica, 1984, pp 50-63.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02181-03 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Pickar	Chief, Section on Clinical Studies	NS, NIMH
Others:	S.M. Paul	Chief	NS, NIMH
	G.A. Roy	Visiting Associate	NS, NIMH
	O.M. Wolkowitz	Medical Staff Fellow	NS, NIMH
	A.R. Doran	Medical Staff Fellow	NS, NIMH
	A.F. Breier	Medical Staff Fellow	NS, NIMH
	J.L. Schreiber	Social Worker	NS, NIMH
	M. Linnoila	Clinical Director	NIAAA

COOPERATING UNITS (if any)

Alcohol Intramural Research Program, National Institute of Alcohol Abuse and Alcoholism; Laboratory of Psychology and Psychopathology, NIMH; Neuropsychiatry Branch, St. Elizabeths Hospital, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to gain a greater understanding of the psychobiology of schizophrenia and to develop improved strategies for the treatment of this illness. Clinical research for this project is carried out on the 4-East Nursing Unit of the Clinical Center. Patients with DSM-III diagnosed schizophrenia are studied during a several month research period during which time they are treated under double-blind conditions with neuroleptic and placebo medications. An important goal of this project has been the investigation of the mechanism of action of neuroleptic drugs and a profile of which symptoms are most responsive to drug treatment. We have observed that neuroleptic-induced, time-dependent decreases in levels of plasma homovanillic acid (HVA) (a major dopamine metabolite) correlate with antipsychotic drug response for a group of schizophrenic patients. Further, we have observed that the degree of reduction of plasma HVA is correlated with the degree of reduction of psychotic symptoms in individual patients. These data suggest that slow to develop neuroleptic effects on dopamine release are related to the clinical effects of neuroleptic treatment. In studies of symptomatology of schizophrenic patients we have demonstrated that both positive and negative symptoms are sensitive to neuroleptic treatment. Results from 24-hour studies in which plasma HVA is sampled hourly have supported our observation of neuroleptic-induced decrease in levels of plasma HVA and have also established a marked diurnal rhythm in plasma HVA in normal controls. The possibility of disordered plasma HVA rhythms in schizophrenic patients is being investigated. We are currently investigating the effects of the novel benzodiazepine, alprazolam, as an additive pharmacologic treatment to neuroleptics in schizophrenic patients.

OTHER PROFESSIONAL PERSONNEL

R.M. Cohen	Chief, Clinical Brain Imaging Section	LPP, NIMH
D. Weinberger	Chief, Section on Clinical Neuropsychiatry	NPB, NIMH

PROJECT DESCRIPTION

This project is part of the research program of the Section on Clinical Studies of the Clinical Neuroscience Branch. This section conducts clinical research based on the 4-East Nursing Unit of the Clinical Center.

Despite enormous research and clinical efforts to alleviate symptoms of schizophrenia, the group of drugs known as neuroleptics introduced over the last two decades have remained as the principle pharmacologic agents for the treatment of schizophrenia. The close relationship between the affinities of representative neuroleptic drugs to bind to non-adenylcyclase dependent postsynaptic dopamine receptors and their clinical antipsychotic potencies represents the foundation of the dopamine hypothesis of schizophrenia. This research program has attempted to study the mechanism of neuroleptic action with particular attention to changes in dopamine system activity as related to clinical response. The goal is to improve our understanding of the pathophysiology of schizophrenia and to provide the foundation for developing improved forms of treatment.

METHODOLOGY

(See: 1984 Annual Report, pp 719-723, Project Number Z01 MH 01281-02 NS, Neurobiology of Schizophrenia)

MAJOR FINDINGS

1. Using our longitudinal study paradigm, we have observed that treatment with the neuroleptic, fluphenazine, induces a time-dependent decrease in levels of plasma HVA. Further, this decrease, occurring after 3 weeks of neuroleptic treatment, correlates with the antipsychotic response to neuroleptic. We have now replicated this initial finding and extended our data to suggest that individual patient antipsychotic response to neuroleptic can be predicted by the patient's decrease in plasma HVA level. Moreover, both positive and negative symptoms show relationships to levels of plasma HVA when patients are drug-free as well as when neuroleptic treated. This pattern of neuroleptic effects on plasma HVA may provide a marker for neuroleptic response and a better perspective into pharmacologic mechanisms of action of this group of drugs.

2. We have continued our studies of CAT scan abnormalities in patients with schizophrenia. Our initial report of enlarged third ventricles in comparison to medical controls is consistent with a larger group of data emerging in the literature. We are currently examining whether patients with enlarged third ventricles respond less well to neuroleptic treatment than do patients with normal ventricular size.

3. We have recently completed a longitudinal study of the sensitivity of positive and negative symptoms of schizophrenic patients to treatment with

neuroleptics. We have observed that both groups of symptoms respond to neuroleptic treatment. We have also noted that the clinical picture with regard to predominance of positive or negative symptoms alters considerably from the drug-free state to the neuroleptic treated state, with an apparent shift towards the emergence of a "deficit" syndrome. These finding have important implications to the field of schizophrenia research with regard to classifying schizophrenic patients into positive and negative types when patients are seen only cross-sectionally while neuroleptic treated.

4. We have completed a therapeutic trial with the calcium channel blocker, verapamil, in schizophrenic patients. Although data analysis is not complete significant clinical effects of this drug were not observed. We did, however, find indication that verapamil gains access to the CNS after oral administration since pharmacologic levels of verapamil were observed in CSF during the fourth week of treatment.

5. We are currently evaluating the use of the novel benzodiazepine, alprazolam, as an adjunctive treatment to neuroleptics in schizophrenic patients. Preliminary data has been particularly promising demonstrating that residual positive and negative symptoms show improvement during alprazolam treatment with a short-term rebound following discontinuation of medication. Further work in this area is continuing.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Schizophrenia is a major public health problem in the United States. This project attempts to study possible etiologic factors in schizophrenia and to develop a better understanding of mechanisms underlying current pharmacologic treatments. Our recent findings with regard to levels of plasma HVA during neuroleptic treatment and their predictive power for neuroleptic response may have considerable importance to the field. Although more research is needed, these findings suggest the possibility that change in plasma HVA may be a marker for the antipsychotic effects of neuroleptic treatment.

Our recent data regarding positive and negative symptoms in schizophrenia has importance to the field of schizophrenia research and to clinical treatment in general. It highlights the importance of evaluating patients in the drug-free condition and gives pause to the notion of classifying patients with regard to positive and negative symptoms when they are seen only during neuroleptic treatment.

Our preliminary data regarding alprazolam is a potentially exciting finding which may have implications for the treatment of schizophrenia. Continued systematic research in this area is needed.

New treatment strategies which may develop from this work would have considerable importance to the field of psychiatry and to the estimated 2 million patients suffering from schizophrenia in the United States. Our approach of studying patients with schizophrenia longitudinally in a research ward setting has proven successful in several areas including the possibility of developing a biological marker for the antipsychotic effects of neuroleptics.

PROPOSED COURSE

1. A major aspect of our program will continue to be examining the usefulness of levels of plasma HVA with regard to predicting the antipsychotic response of patients with schizophrenia. We plan to begin a protocol using the peripheral MAO inhibitor, debrisoquin, in order to deminish peripheral and enhance CNS contributions to levels of HVA measured in plasma. This strategy may help to clarify what contribution to our findings are derived from brain-originated HVA.

2. We will continue to analyze data from 24-hour studies of levels of plasma HVA in schizophrenic patients and normal controls. The possibility of a disordered rhythm in schizophrenic patients or interaction with neuroleptic treatment is an exciting potential finding.

3. We have recently developed methylphenidate and apomorphine infusion strategies in schizophrenic patients in order to better understand dopamine system activity in these patients. During the coming year we expect to examine preliminary data from these strategies.

4. We will continue our examinations of the therapeutic effects of alprazolam as an additive treatment to neuroleptics in schizophrenic patients. This will be accomplished by continued double-blind studies during which time plasma monoamine metabolites will be investigated and examined as possible predictors of clinical response.

PUBLICATIONS

Boronow, J., Pickar, D., Ninan, P. T., Roy, A., Hommer, D., Linnoila, M., and Paul, S. M.: Atrophy limited to the third ventricle in chronic schizophrenic patients: report of a controlled series. Arch. Gen. Psychiatry, 42: 266-271, 1985.

Doran, A. R., Narang, P. K., Meigs, C. Y., Wolkowitz, O. M., Roy, A., Breier, A., and Pickar, D.: Verapamil concentrations in cerebrospinal fluid after oral administration. N. Engl. J. Med., 312(19): 1261-1262, 1985.

Doran, A. R., Pickar, D., Labarca, R., Douillet, P., Wolkowitz, O. M., Thomas, J. W., Roy, A., and Paul, S. M.: Evidence for a daily rhythm of plasma HVA in normal controls but not in schizophrenic patients. Psychopharmacol. Bull., 21: 695-698, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02184-03 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurobiology of Depression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Pickar	Chief, Section on Clinical Studies	NS, NIMH
Others:	S.M. Paul	Chief	NS, NIMH
	G.A. Roy	Visiting Associate	NS, NIMH
	O.M. Wolkowitz	Medical Staff Fellow	NS, NIMH
	A.R. Doran	Medical Staff Fellow	NS, NIMH
	A.F. Breier	Medical Staff Fellow	NS, NIMH
	W.Z. Potter	Chief, Unit on Clinical Psychopharmacology	LCS, NIMH
	D. Rubinow	Chief, Unit on Peptide Studies	BPB, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Clinical Science, Biological Psychiatry Branch and Laboratory of Neurochemistry, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this study is to investigate selected areas of the neurobiology of depression. Depressed patients free of psychotropic medications for at least two weeks are evaluated, diagnosed and symptoms rated on the 4-East Clinical Research Unit of the NIH Clinical Center. During the past year we have attempted to assess neurotransmitter function in depression by measuring levels of amine and amine metabolites in plasma and cerebrospinal fluid (CSF) in depressed patients and normal controls. We have observed higher levels of circulating plasma norepinephrine in unipolar patients with DSM-III diagnosed melancholia in comparison to controls. We have also found that melancholic patients have lower levels of CSF homovanillic acid (HVA) and 3,4-dihydroxy-phenylacetic acid (DOPAC) than nonmelancholic depressed patients although we also observed that depressed patients who have experienced life events prior to the onset of depression tended not to have low levels of these CSF metabolites. These data suggest activation of the sympathetic nervous system and diminished CNS dopaminergic system function, respectively, in patients with melancholia. We have also studied underlying mechanisms involved in the activation of the hypothalamic-pituitary-adrenal (HPA) axis, a disturbance found in many depressed patients. We have observed that nonsuppression to the dexamethasone suppression test (DST) is associated with higher levels of plasma norepinephrine and with higher levels of CSF 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG). We have also found that low levels of the brain peptide, somatostatin, are associated with DST nonsuppression. An experimental paradigm in humans intended to invoke a transitory experience of learned helplessness as a model for depression has also been initiated.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION

The purpose of this project is to investigate selected neurobiological aspects of depressive illness. Towards this end we have studied a spectrum of patients with depression in a short-term intensive program on the 4-East Clinical Research Unit of the NIH Clinical Center. After at least 14 days free of all medications, patients are studied using a variety of methodologies including the assessment of levels of monoamine and monoamine metabolites in plasma and cerebrospinal fluid, and investigations of biological mechanisms underlying hyperactivity of the hypothalamic-pituitary-adrenal axis in depression. We have also attempted to develop a model for learned helplessness in humans.

METHODOLOGY

(See: 1984 Annual Report, pp 725-728, Project Number Z01 MH 02184-02 NS, Neurobiology of Depression)

MAJOR FINDINGS

1. We have observed that depressed patients who have experienced a life event during the 6 months prior to the onset of their illness showed "normal" levels of CSF amine metabolites in contrast to the low levels of CSF HVA in depressed patients who had not experienced a life event. Continuing work examining suicidal behavior in CSF amine metabolites has revealed a tendency for lower levels of CSF HVA and 5-HIAA in patients with histories of suicide attempts.

2. Continuing research is focused on HPA axis function in depression. Consistent with our previous findings, levels of plasma MHPG are found to be higher in depressed patients who do not normally suppress cortisol to dexamethasone administration. These and our previous findings are consistent with the notion of an associated hyperactivity of a noradrenergic and HPA axis in subgroups of depressed patients. We have also observed that low levels of the peptide, somatostatin, in the CSF of patients with depression or schizophrenia are associated with non-suppression to dexamethasone.

3. During the past year we have developed an experimental paradigm intended to elicit a transitory period of learned helplessness in human subjects. This model involves the presentation of noise stimuli to the subjects; under one condition they are able to learn how to turn off the noise (escape) and in a paired experimental condition are unable to stop the noise stimuli (nonescape). Preliminary results in a group of normal controls suggest enhanced cortisol and ACTH response to the nonescape stimuli, with indication of subjective assessment of depressed mood. Further studies using this paradigm in normal controls and depressed patients are in progress.

4. We have studied the biochemical effects of the administration of the steroid dexamethasone. Our observation of dexamethasone-induced increase in levels of plasma HVA in normal subjects is consistent with preclinical studies of steroid-dopamine interaction. Prospective investigation into the behavioral and biochemical effects of prednisone administration is currently in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

In this project we have focused on selected areas of neurobiology pertaining to depressive illness. Our findings continue to support an association between noradrenergic and HPA axis dysregulation in some depressed patients. The difference between levels of CSF amine metabolites in patients who have and have not experienced a life event is a new finding with potentially important ramifications for understanding environmental interactions with the underlying neurobiology of depression. The developing model of learned helplessness in humans is important since it may provide a selective stimuli to uncover biological dysfunction in depressed patients.

PROPOSED COURSE

We will continue to study medication-free depressed patients with regard to CSF and plasma amine metabolites. We intend to focus effort on studying depressed patients in the learned helplessness paradigm, specifically examining stress-related differences between controls and levels of plasma cortisol, ACTH, and in amine metabolites.

PUBLICATIONS

Doran, A. R., Rubinow, D. R., Roy, A., and Pickar, D.: CSF somatostatin and abnormal response to dexamethasone administration in schizophrenic and depressed patients. Arch. Gen. Psychiatry (in press).

Roy, A., Breier, A., Doran, A. R., and Pickar, D.: Life events in depression: relationship to subtypes. J. Affective Disord. (in press).

Roy, A., Pickar, D., Linnoila, M., Doran, A. R., Ninan, P., and Paul, S. M.: Cerebrospinal fluid monoamine and monoamine metabolite concentrations in melancholia. Psychiatry Res. (in press).

Roy, A., Pickar, D., Linnoila, M., Doran, A. R., and Paul, S. M.: Cerebrospinal fluid monoamine and monoamine metabolite concentrations and the dexamethasone suppression test in depression: relationship to life events: Arch. Gen. Psychiatry (in press).

Roy, A., Sutton, M. S., and Pickar, D.: Neuroendocrine and personality variables in dysthymic disorder. Am. J. Psychiatry, 142(1): 94-97, 1985.

Wolkowitz, O. M., Sutton, M. E., Doran, A. R., Labarca, R., Roy, A., Thomas, J. W., Pickar, D., and Paul, S. M.: Dexamethasone increases plasma HVA but not MHPG in normal humans. Psychiatry Res. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02187-02 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diazepam Infusions as a Measure of Benzodiazepine Receptor Sensitivity in Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.W. Hommer	Staff Psychiatrist	NS, NIMH
Others:	S.M. Paul	Chief	NS, NIMH
	D. Pickar	Chief, Section on Clinical Studies	NS, NIMH
	O.M. Wolkowitz	Medical Staff Fellow	NS, NIMH
	A. Breier	Medical Staff Fellow	NS, NIMH
	H. Weingartner	Chief, Section on Psychopathology	LPP, NIMH
	V. Matsuo	Physiologist	IR, NEI

COOPERATING UNITS *(if any)*

Laboratory of Psychology and Psychopathology, NIMH; Intramural Research, National Eye Institute; Alcohol Intramural Research Program, National Institute of Alcohol Abuse and Alcoholism; Tufts University

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Increasing doses of the benzodiazepine, diazepam, or placebo were administered to normal volunteers or drug-free alcoholic inpatients in a double-blind, cross-over study. In one series of experiments, subjects were pretreated with either placebo or the benzodiazepine antagonist, Ro-15-1788. In another series, subjects were pretreated with placebo or a high or low dose of caffeine. Following each dose of drug saccadic eye velocity, diazepam blood levels, plasma cortisol, and growth hormone were measured and self-ratings of anxiety and sedation were performed. After every other dose cognitive testing of memory and attention was performed. The effects of diazepam on these variables was quantified and diazepam dose response curves constructed. These dose response curves provide a measure of benzodiazepine receptor sensitivity in humans, as well as an evaluation of the ability of specific and nonspecific antagonists to block the actions of benzodiazepines.

OTHER PROFESSIONAL PERSONNEL

M. Linnoila	Clinical Director	ALC, NIAAA
D. Greenblatt	Tufts University School of Medicine	

PROJECT DESCRIPTION

We have been examining the ability of two BZ antagonists, the specific antagonist Ro-15-1788 and the non-specific antagonist caffeine, to block the effects of intravenous diazepam. Ro-15-1788 is given i.v., 0.035 mg/kg. Caffeine is given orally either 3 or 10 mg/kg. Then diazepam is administered intravenously in doses of 25, 25, 50, and 100 μg/kg at 15 minute intervals. After each dose measurement of a variety biological and psychological variables that are affected by benzodiazepines is made. These variables include: memory and attention (done with Dr. Herbert Weingartner), velocity of saccadic eye movements (done with Dr. Victor Matsuo of the National Eye Institute), growth hormone, cortisol, and self-ratings of anxiety and sedation.

MAJOR FINDINGS

Plasma cortisol, saccadic eye velocity, self-rated sedation and recent memory all significantly decrease during diazepam administration while growth hormone concentration is increased significantly in most, but not all, individuals. From this data we constructed diazepam dose-response curves. These dose-response curves provide an in vivo measure of BZ receptor sensitivity in humans. Furthermore, we found extremely high correlations between all these variables during diazepam administration suggesting that these disparate effects are all mediated through the same class of BZ receptors as well as a high correlation between changes in these variables and increasing plasma concentrations of diazepam.

In addition to studying BZ receptor sensitivity in normals we have also examined BZ receptor sensitivity in five chronic alcoholic subjects who had been alcohol and drug-free for one month at the time of the study. These subjects showed a greater sensitivity to diazepam than any of the normal controls. We have also found that caffeine and Ro-15-1788 both block the effects produced by diazepam although the various effects produced by BZ's seem to vary in their sensitivity to blockade by caffeine. The effects of diazepam on eye-movements and sedation are most effectively antagonized by pretreatment with caffeine while the effects of diazepam on memory are least affected. Ro-15-1788 appears to block all the effects of diazepam equally well.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Benzodiazepines are the most commonly prescribed psychotropic drugs in the world. The recent identification of stereospecific receptors for BZs in the brain of both animals and humans raises several new and exciting avenues of research not only into the mechanism of action of these anxiolytic and anticonvulsant agents but also into the pathophysiology of anxiety itself. For example, studies in rats have demonstrated that stress changes brain BZ receptors. If a similar phenomena occurs in humans it may provide the etiological link between stress and mental illnesses such as the anxiety,

depression and post-traumatic stress disorder. In order to study BZ receptor sensitivity in psychiatric illness it is first necessary to develop a valid and reliable measure of BZ receptor sensitivity in normal human subjects. This project has provided such a measure of BZ receptor sensitivity and our preliminary results suggest that BZ receptor sensitivity may be altered in alcoholism.

PROPOSED COURSE

Further studies of BZ receptor sensitivity in various psychiatric disorders including anxiety and depressive disorders are planned. We also plan to study the effects of stress on BZ receptor sensitivity.

PUBLICATIONS

Hommer, D. W., Matsuo, V., Wolkowitz, O. M., Chrousos, G., Greenblatt, D. J., Weingartner, H., and Paul, S. M.: Benzodiazepine sensitivity in normal human subjects. Arch. Gen. Psychiatry (in press).

Wolkowitz, O. M., Weingartner, and Hommer, D. W.: Specificity of benzodiazepinee disruption of memory. Presented as New Research at APA Annual Meeting, Dallas, TX, 21 May 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02188-02 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological Studies of Borderline Personality Disorder

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.L. Gardner	Staff Psychiatrist	NS, NIMH
Others:	R.W. Cowdry	Clinical Director	NIMH
	K. O'Leary	Social Worker (Research)	OD, DIRP, NIMH
	R.L. Post	Chief	BP, NIMH
	M. Kling	Medical Staff Fellow	BP, NIMH
	R. Coppola	Senior Engineer	LPP, NIMH

COOPERATING UNITS *(if any)*

Office of the Director, Division of Intramural Research Programs, NIMH; Biological Psychiatry Branch, NIMH; Laboratory of Psychology and Psychopathology, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with borderline personality disorder and rejection-sensitive dysphoria participated in a program of clinical and biological evaluation and psychopharmacologic treatment. In addition to having labile moods and behavioral dyscontrol, these individuals were found to have a high incidence of psychosensory symptoms similar to those seen in complex partial seizures. A high incidence of non-specific electroencephalographic (EEG) abnormalities and gross structural abnormalities on computerized axial tomography (CT) scans was previously reported; collection and analysis of data from a control population is underway. Procaine infusions frequently produced dysphorias similar to those occurring naturally, and activated a high frequency band of EEG activity over the temporal lobes, which showed a strong positive correlation with the degree of dysphoria, but no correlation with drug response.

A year-long, double-blind, placebo-controlled cross-over study of the effects of medication on mood and dyscontrol behavior suggested that alprazolam (a triazolobenzodiazepine) may disinhibit these individuals, resulting in behavioral dyscontrol; that low-dose trifluoperazine (an antipsychotic), is not consistently different from placebo; that carbamazepine (an anticonvulsant) significantly decreases dyscontrol and produces improvement in physician (but not patient) ratings; and that tranylcypromine (a monoamine oxidase inhibitor with antidepressant actions) produced significant global improvement in mood and had variable effects on behavioral dyscontrol. An analysis of the data for predictors of drug response, conducted this past year, disclosed few predictors.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION

Rejection-sensitive or hysteroid dysphoria is a poorly understood syndrome occurring in many individuals with a diagnosis of borderline personality disorder. This syndrome, described by Klein and others, is characterized by the rapid onset of a dysphoric mood (sometimes characterized more specifically by depression, anxiety, or rage) following an actual, threatened, or imagined rejection. Behavioral dyscontrol is not uncommon, involving violence, direct injury to self, or overdosage with sedating medications. This disorder accounts for a significant number of admissions to short-term psychiatric units, and is one of the more difficult disorders treated in long-term outpatient psychotherapy.

There are a number of theories about the etiology of the borderline personality in general, and rejection-sensitive dysphoria in particular, most emphasizing developmental psychodynamics. Pathological parent-child interactions, particularly involving a failure of empathy during the symbiotic period or a failure of the separation-individuation process have been described as etiologic factors. Kernberg, while emphasizing the developmental abnormalities of this disorder, speculates that constitutional factors, such as an excess of aggressive drive, contribute to its development. However, very little research has been done to validate these hypotheses, or to explore possible biological mechanisms, such as abnormalities in limbic system functioning.

This project consist of two parts: (1) a clinical and biological evaluation, including symptomatology, developmental variables, neurophysiological function (EEG's and procaine-activated EEG's), and central nervous system structure (CT scans), and (2) trials of psychopharmacologic agents.

MAJOR FINDINGS

Phenomenology

An article was published this year describing in detail the phenomenology of the dysphoric states experienced by borderline patients, the associated self-injury, and possible underlying biological predispositions. The dysphoric episodes are commonly precipitated by specific emotional stimuli (such as perceived rejection and are generally relatively brief, although if the perceived stimuli continue they may develop into prolonged regression. These dysphorias may be accompanied by self-injurious acts such as wrist cutting, cigarette burns, arm and head banging, drug overdoses, and drug and alcohol abuse. Clinical descriptions from our twenty patients plus detailed written descriptions from five patients show that these episodes of behavioral dyscontrol can be differentiated from true suicidal acts, an important distinction in determining the proper therapeutic approach, and suggest the presence of an underlying biological mechanism which contributes to the escalation of the dysphoria to acts of dyscontrol. This response to rejection raises questions of biological vulnerability to recurrent psychic stress, consistent with Post's "kindling model" and Ademac's findings of heritable differences in limbic excitability to kindlings in animals. In addition, 50% of our patients report an absence of pain during self-injurious behavior, suggesting alteration in pain mechanisms mediated by the endogenous

opiate system. Continued phenomenological examination of responses to rejection and loss will further illuminate these dysphoria-related parasuicidal acts.

Electroencephalography (EEG) and Computerized Tomography

The symptomatology shows a striking overlap with that of complex partial seizure (CPS). The behavior is similar to that described as episodic dyscontrol (ED). Since both CPS and ED have associated electroencephalographic abnormalities probably related to dysfunction of limbic or diencephalic structures, we hypothesize that the borderline syndrome also has a distinctive neurophysiological substrate involving limbic and diencephalic dysfunction. Abnormalities observed in elechencephalographic studies and computerized tomography of the cerebrum were summarized in the previous annual report. Routine and computer analyzed EEG's are being conducted on age-matched controls for comparison to patients in the study. CT scans of patients will be compared to existing normal control data and to patients with schizophrenia.

Procaine-Activated EEG's

Procaine-activated EEG's have been obtained in 17 patients and 10 normal volunteers to date, incollaboration with Drs. Robert Post and Mitch Kling (BPB) and Dr. Richard Coppola (LPP). Clinically, all patients had noteworthy transient responses, including dramatic dysphoria, euphoria, paranoia, fear or a sense of impending death. These responses tended to closely approximate the spontaneously occurring dysphorias experienced by these patients. Olfactory hallucinations and auditory perceptual distortion were commonly reported In contrast, normal volunteers did not experience dysphoria but reported physical sensations (rapid heart rate, buzzing in their ears, tingling in extremities) as the predominant reaction. Two volunteers reported a transient euphoric response (one compared it to a sexual organism) and four reported a calming effect. Overall, responses in the normal volunteers were less intense and more short-lived than described by the patients.

During the procaine infusion, EEG's have been recorded using a 16 channel left-hemisphere montage developed by Dr. Richard Coppola (LPP). The spectral analyses of both these EEG recordings demonstrate that procaine activates a high frequency (40-50 Hz) EEG band in the anterior and mid-temporal lobe regions, and suggest that the activation may occur most prominently in patients with rejection-sensitive dysphoria and behavioral dyscontrol.

Blood samples have also been collected during the procaine infusions for neuroendocrine assays (ACTH, cortisol, prolactin). Preliminary analysis of this data suggest a correlation between dysphoric response to procaine and marked elevation of ACTH levels.

Treatment: Trials of psychopharmacologic agents

Results of the medication trials, as reported in the previous annual report, showed that carbamazepine, an anticonvulsant, and tranylcypromine, an anti-depressant, were efficacious in treating aspacts of borderline personality disorder. Carbamazepine seemed most effective in reducing the episodes of dyscontrol and impulsivity. Tranylcypromine proved to be an effective

antidepressant with some reduction in emotional lability. Alprazolam, an antianxiety agent, while lessening anxiety, was associated with an increased incidence of dyscontrol. Trifluoperazine, also effective as an antianxiety agent in this disorder, otherwise was no more effective than placebo.

Analysis during the past year has been directed towards identifying possible predictors of drug response. This analysis included symptomatology, past history of affective disorders, neurological soft signs, EEG findings, CT scan findings, dyscontrol behavior, family history of psychiatric disorder, and response to other medications. Few predictors were found. Carbamazepine appeared to be for effective in individuals with more severe illness, and positive behavioral response to carbamazepine was most prominent in individuals with abnormal EEG's. Tranylcypromine response was most marked in individuals with a history suggestive of attention deficit disorder in childhood, a result consistent with the amphetamine-like actions of tranylcypromine. In addition, further analysis failed to show a connection between the clinical or EEG response to procaine infusion and medication response. Further delineation of the varied manifestations of this disorder should enhance our ability to predict and individualize medication responses.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Rejection-sensitive dysphoria and borderline personality disorder are common disorders, particularly in the young adult population. They account for a significant number of short-term psychiatric hospitalizations and are frequently associated with major, often life-threatening overdoses, with self-multilation, and with episodes of violence. The etiology of these disorders is a matter of great controversy and limited data, as is the role of medication in the treatment of these individuals.

The evaluation phase of this study provides tentative support for a theory of these disorders which emphasizes an interaction between developmental traumata and biological predisposition. If further studies confirm the association between low threshold for dysphoria and dyscontrol on the one hand and procaine induced high frequency EEG activity over the temporal lobe on the other, the line between limbic system abnormalities and labile mood and impulsive behavior is strengthened. Specific pharmacologic strategies for altering the responsivity of limbic system structures may ameliorate the dysphorias, may lessen the likelihood of dyscontrol, and may enhance the usefulness of psychotherapy in this disorder.

As previously noted, there is little evidence from controlled trials regarding the usefulness of medication in this disorder. The finding of significant clinical benefits from both an anticonvulsant and an antidepressant is important in itself, but suggests the value of further biomedical research to elucidate possible links between the biological underpinnings of this disorder and the biological mechanisms involved in mood disorders and in epileptic or epileptoid disorders.

PROPOSED COURSE

Further clinical, developmental, and biological data are needed from a larger number of patients. The next generation of studies will focus on an intensive

evaluation of phenomenology, cognitive functioning, neurophysiological and neuroendocrine variables, and neurochemical systems, with comparison of these findings to normals as well as other psychiatric diagnostic subgroups. In particular, EEG, CT, and PET scanning will focus on neuroanatomical and neurophysiological questions with specific interest directed to issues of laterality. The procaine EEG will be expanded and potentially coupled with PET scanning to explore possible visualization of brain structures involved in the dysphoric process. A series of controlled infusin studies are planned to investigate several of the neurochemical systems. Because of the history of altered pain mechanisms, naloxone infusions will be utilized to investigate the endorphin system. While these patients often report brief psychotic episodes and trends toward paranoia, they also report calming effects from former use of stimulants. Methylphenidate infusions will assess clinical and monoaminergic responses to this specific stimulant. Clinical responses to methylphenidate may prove useful in predicting future antidepressant responses. Panic attacks, generalized anxiety, and phobias are not uncommon in this disorder and we plan to include an infusion of yohimbine, an alpha adrenergic agonist, to stimulate the noradrenergic system and compare the findings to patients who have anxiety disorders without the accompanying personality disorder. These studies are designed to aid in further delineation of the complex symptomatology of this disorder. These studies may provide further data to help predict response to medication, which would provide a controlled empirical base for decisions which are currently highly subjective.

PUBLICATIONS

Gardner, D. L. and Cowdry, R. W.: Alprazolam-induced dyscontrol in borderline personality disorder. Am. J. Psychiatry, 142(1): 98-100, 1985.

Gardner, D. L. and Cowdry, R. W.: Article-induced dyscontrol. Am. J. Psychiatry, 142(6): 776, 1985.

Gardner, D. L. and Cowdry, R. W.: Suicidal and parasuicidal behavior in borderline personality disorder. Psychiatr. Clin. North Am., 8(2): 389-403, 1985.

Cowdry, R.W., Pickar, D. and Davies, R.: Symptoms and EEG findings in the borderline personality syndrome. Int. J. Psychiatry Med. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00117-10 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alpha-Adrenergic and Prostaglandin Receptors in Human Blood Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. S. Kafka	Physiologist	NS, NIMH
Others:	J. Nurnberger	Psychiatrist	CNG, NIMH
	A. Roy	Psychiatrist	NS, NIMH
	T. Uhde	Psychiatrist	BP, NIMH
	R. Polinsky	Neurologist	CN, NINCDS

COOPERATING UNITS *(if any)*

Section on Clinical Studies, NS, NIMH; Clinical Neuropharmacology, NINCDS; Unit on Anxiety and Affective Disorders, BP, NIMH; Clinical Neurogenetics Branch, NIMH.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alpha-Adrenergic receptors and prostaglandin receptors were studied in human blood cell preparations. The ability of adrenergic agonists to inhibit adenylate cyclase was used as a measure of the alpha$_2$-receptors' biological function. Binding of tritiated alpha-adrenergic receptor antagonists to membrane receptors on platelets was measured. These measures were correlated and are used to assess alpha 2 adrenergic physiological function in normal individuals, patients with various psychiatric disorders, and diseases of neurotransmission and patients receiving different psychopharmacologic agents.

Project Description:

Objectives

(1) To study alterations in α-adrenergic receptor number and function in psychiatric diseases and other pathological conditions characterized by altered adrenergic transmission. To measure changes in receptor number and function with different treatment modalities.

(2) To provide an experimental model for the study of drug-induced or physiologically-induced changes in central receptor sensitivity in man.

(3) To understand the cellular events connecting receptors, occupied by their agonists, with the activity of the adenylate cyclase enzyme complex and subsequent physiological events.

Methods Employed:

(See: 1984 Annual Report, pp. 739-742, Project Number Z01 MH 001171-09 NS, Alpha-Adrenergic and Prostaglandin Receptors in Human Blood Elements.)

Major Findings:

1) In patients with orthostatic hypotension, the platelet findings differed with orthostatic hypotension of differing etiologies. Platelets from patients with idiopathic orthostatic hypotension or multiple system atrophy had more α_2-receptors than platelets from control subjects. Platelets from patients with idiopathic orthostatic hypotension, but not multiple system atrophy, produced less PGE_1,-stimulated cAMP. Platelets from patients with sympathotonic orthostatic hypotension were similar to platelets from controls in both measures. The percent NE-inhibition of cAMP production was similar in platelets from orthostatic hypotensive patients and controls.

2) ^{3}H-dihydroergocryptine binding to platelets from depressed patients was increased compared with platelets from control subjects. PGE_1-stimulated cAMP production and NE-inhibition of PGE_1-stimulated cAMP production were decreased in platelets from depressed patients. Receptor binding and functional responsiveness are dissociated in these patients, suggesting the possibility that increased binding is secondary to a defect in α_2-receptor responsiveness. Perhaps the increase in α_2-receptors is due to uncoupling of the receptors from the adenylate cyclase complex. Alternatively, the increased number of α_2-receptors may be secondary to decreased circulating NE. As the plasma NE concentration was not different in depressed patients and normal subjects, the second possibility may be less likely.

The number of binding sites, in any case, may not reflect the level of responsiveness of the α_2-receptor. If neurons share with platelets the changes observed in depressed patients, it is possible that PGE_1-stimulation of cAMP and the functional responsiveness of the α_2-adrenergic receptor are decreased in depressed patients.

3) In a small group of obsessive-compulsive patients (n=8), platelets had increased binding to α_2-receptors and diminished PGE_1-stimulated cAMP production, as well as blunted NE-inhibition of PGE_1-stimulated cAMP production. The sample size was too small for a meaningful statistical comparison with controls, but the data were similar to platelet findings in panic/agoraphobic and in depressed patients, both of which groups were significantly different from controls. The data suggest that other tricyclic-responsive groups may share with patients with affective disorders a reduced responsiveness of the α_2-receptor. The increase in binding to α_2-receptors may be compensatory to this deficit, as discussed above. Perhaps tricyclic antidepressants increase the efficiency of α_2-receptor coupling to the cAMP complex, compensating for the defect. It is possible that such compensation plays a role in the therapeutic effects of antidepressants.

4) Platelet cAMP production is insensitive to stimulation in patients with hypokalemic syndromes. Perhaps certain K^+ levels essential to cAMP production.

5) Schizophrenic quadruplet sisters (Genain family) show the α_2-receptor function platelet changes observed in other schizophrenic patients. Close relatives also have some of the platelet alternatives, suggesting a possible genetic component in this marker.

6) Clorgyline treatment of depressed patients does not alter their platelet α_2-receptor function, suggesting that clorgyline does not act directly on the α_2-receptor.

Significance to Biomedical Research and to the Program of the Institute

The results obtained from these experiments are directly applicable to an assessment of receptor function in human disease states, and to monitoring the physiological effects of various drug treatments. If peripheral α_2-adrenergic receptors are related to, or can serve as models for the central nervous system α_2-receptors, valuable information about the function of these central receptors in psychiatric diseases may be obtained. It is possible that α_2-adrenergic receptor function in central and peripheral nervous systems is similar to that measured in platelets. Different platelet defects accompany orthostatic hypotension of different etiology, suggesting the usefulness of the platelet measure in studying alterations in hypotension with treatment. For example, platelets in idiopathic and multiple system atrophy hypotension differ in their defects, while in sympathotonic orthostatic hypotension, which is not characterized by peripheral blood vessel or baroreceptor defects, the platelet measures are like control. Longitudinal measurement of platelet α_2-function might indicate which parts of the sympathetic nervous system are normalizing with a given treatment.

In depressed patients, and perhaps in patients with obsessive-compulsive disorder and panic disorder with agoraphobia, there may be a defect in α_2-receptor coupling to the cAMP complex. The efficacy of antidepressants in correcting the defect might provide a means of evaluating the efficacy of that drug in treating the particular patient. The comparison of α_2-receptor number during treatment,

if increased α_2-receptor number is a secondary concommitant of receptor uncoupling, might provide a relatively simple means of monitoring treatment efficacy, especially if a singe B_{max} value for binding to the α_2-receptor in blood could be shown adequate for use in monitoring treatment.

Proposed Course

The Principal Investigator is on an extended detail and the project will be suspended indefinitely.

Publications

1) Siever, L.J., Kafka, M.S., Insel, T.R., Lake, C.R., and Murphy, D.L. "Effect of long-term clorgyline administration on human platelet - adrenergic receptor binding and platelet cyclic AMP responses" Psychiatry Res 9:37-44 (1983.

2) Kafka, M.S., Polinsky, R.J., Williams, A., Kopin, I.J., Lake, C.R., Ebert, M.H., and Tokola, N.S.: "Alpha-adrenergic receptors in orthostatic hypotension syndrome". Neurology, 34:1121-1125 (1984).

3) Siever, L.J., Kafka, M.S., Targum, S. and Lake, C.R. "Platelet - adrenergic binding and biochemical responsiveness in depressed patients and controls." Psychiatry Res. 11:342-302.

4) Gullner, H.G., Kafka, M.S., Gill, J.R.Jr., "Insensitivity of platelet adenylate cyclase to prostaglandin E, in patients with hypokalemic disease" Annals of Internal Medicine 101:342-343 (1984).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00159-06 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurotransmitters Receptors in the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.S. Kafka Physiologist NS, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rat brain neurotransmitter receptors were studied. There were circadian rhythms in neurotransmitter receptors in discrete brain areas circadian rhythm was present in cyclic AMP production in cerebral cortical slices, as a functional response to occupation of α) and β-adrenergic receptors. There was a circadian rhythm in arginine vasopresser in the suprachiasmatic nuclei.

Project Description:

Objectives:

(1) To develop methods to measure the presence of neurotransmitter receptors in the nervous system.

(2) To measure whether alterations in receptor number accompany alterations in function in the central nervous system.

(3) To assess whether there are circadian rhythms in functionally significant responses to receptor activation.

(4) To measure whether there are circadian rhythms in catecholamine secretion in discrete brain regions, and what their relationship is to circadian rhythms in cAMP and cGMP.

Methods Employed:

The specific binding of tritiated ligands to membranes prepared from rat brains is used to measure rhythmic changes in receptor binding in rats sacrificed at intervals over a 24-hour period. The α_1-receptor is measured by the binding of ^{3}H-prazosin; the β-adrenergic receptor, by ^{3}H-dihydroalprenolol; the muscarinic acetylcholine receptor by ^{3}H-QNB; the benzodiazepine receptor by ^{3}H-flunitrazepam; and the α_2-receptor by ^{3}H-para-aminoclonidine. Methods to measure binding in very small tissue samples were devised.

Major Findings:

(1) There are circadian rhythms in a number of receptors in many discrete brain regions measured. The peak binding to α-adrenergic receptors are usually in the dark part of the day when rats are active.

(2) There are circadian rhythm in catecholamine metabolates, cAMP, and cGMP in discrete brain regions.

Significance to Biomedical Research and the Program of the Institute:

Previous work documented the existence of circadian rhythms in neurotransmitter receptor density. These rhythms changed with chronic psychoactive drug administration. More recent studies examined whether circadian rhythms in receptors were functionally significant. In vitro, the circadian rhythm in the number of α_1-and β-adrenergic receptors stimulated by NE can regulate the circadian rhythm in cAMP production. Perhaps the number of adrenergic receptors stimulated by NE modulates the magnitude of cAMP production in situ. As the intraneuronal cAMP concentration is thought to act as a second messenger, regulating the phosphorylation and activation of cellular proteins, circadian rhythmic changes in neuronal cAMP production could have a profound effect on neuronal activity and neuronal transmission across the day.

Proposed Course:

The Principal Investigator is on an extended detail and the project will be suspended indefinitely.

Publications:

1) Kafka, M.S.: "The Effects of antidepressants on circadian rhythms in brain neurotransmitter receptors." Acta Pharmacologica et Toxicologica 56 (Supplement) 162-169 (1985).

2) Kafka, M.S., Marangos, P.J., and Moore, R.Y., "Suprachiasmatic nucleus ablation abolishes circadian rhythms in rat brain neurotransmitter receptors" rain Research 327: 344-347 (1985).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00179-04 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological Aspects of Peptides in Mammalian Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Skirboll	Pharmacologist	NS, NIMH
	D. Hommer	Staff Psychiatrist	NS, NIMH
Others:	E. Mezey	Visiting Associate	LCB, NIMH
	B. Robertson	Guest Researcher	NS, NIMH
	A. Kiss	Visiting Fellow	NS, NIMH

COOPERATING UNITS (if any)

Laboratory of Cell Biology, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using immunohistochemical techniques in combination with retrograde tracing, we have examined the inntervating transmitters of several brainstem nuclei, including peptides, catecholamines and indoleamines. As a continuation of our interest in the hypothalamus, we have gathered evidence on an NPY-adrenaline projection from the brainstem to the paraventricular nucleus (PVN). Evidence has also been obtained that a subset of the corticotropin releasing factor (CRF) neurons in the PVN coexist with cholecystokinin (CCK). In addition, VIP projections in the hypothalamus have been examined with regard to projections from the suprachiasmatic nucleus to the median eminence. In another series of studies, we have confirmed a coexisting Substance P-serotonin projection to one area responsible for central respiratory control, the phrenic motor nucleus. In dorsal root ganglia, evidence has been obtained to suggest co-staining of a subpopulation of neurons with both carbonic anyhydrase and cholera toxin. Finally, ongoing studies are exploring: the coexistence of dynorphin and GABA; a complete mapping of the stria terminalis; and cholinergic projections to the mesencephalon.

Projection Description
Objectives:

In recent years, a growing number of peptides have been identified in the central nervous system. The discovery of neuropeptides has raised many questions regarding their localization, source, mechanism of action, function and fate. A great deal of effort has been devoted to localize them topographically in various brain regions. Mapping of the distribution of neuropeptides is a joint achievement of biochemistry and neuroanatomy. Biochemical mapping studies, however, give no information about the cellular distribution of neuropeptides in the central nervous system. The immunohistochemical method is of value in delineating localization of peptide containing cell bodies in which synthesis could potentially occur, neuropeptide containing fibers and pathways as well as localizing peptides in nerve terminals.

Recent years have also seen the development of new anatomical techniques to trace nerve projections. Both anterograde and retrograde studies have made use of the ability of neurons to take up markers into their neurons, incorporate them into specific compartments and transport them. These techniques permit the mapping of the pathway but offer no information regarding the specific neurotransmitter present. More recently, the development of a technique in which retrograde tracing can be combined with immunohistochemistry permits transmitter-specific pathway tracing in brain.

Methods Employed:

During the past year our laboratory has been using several techniques to further elucidate transmitter specific pathways. These include: indirect, avidin-biotin and PAP immunohistochemistry. These methods are based on the principle of utilizing specific antibodies as a means of localizing tissue antigens, and the antigen-antibody complex is visualized in neuronal perikarya, fibers or nerve endings. In addition, retrograde tracing methods employing agents such as fluorescent dyes, wheat germ agglutinin and phaseolus vulgaris leucoagglutinin were used to examine specific pathways. Finally a method is being developed for identification of a specific transmitter group using NADPH-diaphorase histochemistry.

Briefly, in immunohistochemical experiments, animals are first treated with colchicine to block axonal transport of peptide and thus accumulate antigen in the cell body. This is followed by tissue preparation in which animals are perfused with a paraformaldehyde fixative, brains are sectioned on a cryostat and sections are incubated with a primary antibody. In indirect immunofluorescence, sections are subsequently rinsed, stained with a secondary antibody which has a fluorescence molecule attached and visualized in the fluorescent microscope. In avidin-biotin immunohistochemistry, after primary antibody incubation, sections are incubated with biotin, the ABC complex, developed with DAB and osmium and finally visualized under the bright field microscope.

In a second series of specific experiments, transmitter specific tracing of brain pathways was accomplished using retrograde tracing methods. Briefly, fast blue

or true blue (fluorescent dyes) are injected into nerve terminal areas of interest allowed to migrate to cells of origin. In this manner, dye is taken up into axons and transported back to the cells of origin and visualized in the fluorescent scope. Once the dye is viewed, these same sections can be stained for immunohistochemical procedures as described above, thus permitting transmitter-specific mapping of neuroprojections. Similarly, in retrograde experiments, wheat germ agglutinin conjugated with HRP or in anterograde experiments phaseolus leucoagglutinin are injected into appropriate areas and subsequently developed by DAB intensified with cobalt or nickel ammonium sulfate, respectively, and visualized under the bright field microscope.

Finally, NADPH-diaphorase activity is visualized by incubating sections in NADP-tris, nitroblue tetrazolium and sodium maleate, rinsed in PBS and mounted. Cells visualized in the bright field scope using this method have been shown to be acetylcholine containing. (see MH-02180-02).

Major Findings:

In one series of studies, we have been examining the cells and innervating transmitters of several brainstem nuclei including the nucleus of the solitary tract and the ambiguous nucleus. Specifically, catecholamine somata and terminals were examined using antibody to tyrosine hydroxylase with PAP immunostaining procedures. In some experiments, double immunohistochemistry using the avidin-biotin methods was applied to simultaneously visualize somata producing biogenic catecholamines and indoleamines. As a continuation of our interest in the hypothalamus and its multitransmitter control of endocrine functions (MH-02180-02), we continued to look at the presence of specific transmitter groups in this area. In one series of studies, phaseolus vulgaris was applied iontophoretically to the suprachiasmatic nucleus to examine the projection of this system to the median eminence. Subsequent injections of wheat germ agglutinin into the median eminence permitted retrograde investigations of VIP immunostained somata in the caudal hypothalamus.

In studies of the paraventricular nucleus (PVN), we examined the role of innervating transmitters on immunostaining in the PVN. We have shown that there is a dense epinephrine innervation of the parvocellular subdivision of PVN and that these cells are primarily corticotropin releasing factor (CRF) containing. In addition, we have reported an overlap between the epinephrine fibers and fibers staining for NPY. In more recent studies, using retrograde transport of fluorescent dyes we have confirmed that it is the NPY-adrenaline cells of the C1 and C2 area of the medulla which project to and appear to innervate the parvocellular group of the PVN. In addition, we have shown that the CRF-containing neurons of the PVN also stain for cholecystokinin (CCK) in a subpopulation of neurons suggesting that CCK may play a role in the regulation of ACTH release. Finally, wheat germ agglutinin was injected into the PVN with the aim to investigate the possible uptake of HRP marker granules to the suprachiasmatic nucleus.

We have earlier confirmed a projection from the phrenic motor nucleus to the diaphram in the cat. We have now extended these studies to the rat, in which

retrogradely labelled cells were located in the ventral horn of the 4th and 5th cervical spinal cord segment after dye injection into the diaphragm. These sections were subsequently stained for a variety of antibodies including positive findings for Substance P (SP), thyrotropin releasing hormone (TRH) and serotonin (5-HT). These findings suggested that coexisting cells in the medulla may project to the phrenic motor nucleus. These studies were then extended to include dye injections into the ventral horn of the spinal cord appropriate to the location of phrenic projections and examination of the brainstem raphe nuclei. Brainstem sections showing retrogradely labelled cells were processed with antibodies to SP and 5-HT to reveal that the same neurons which are retrogradely labelled also stain for these specific antigens. These findings suggest that the SP-5-HT coexisting system in the raphe medulla project to the phrenic motor nucleus and thus may control and/or modulate information relay to the diaphragm to control breathing.

Recently, Hokfelt and coworkers have reported a dynorphin projection from the caudate nucleus to the substantia nigra, zona reticulata. The main pathway governing this connection has been known for some time to be GABA. Because of our interest in the function of this basal ganglia feedback (see MH-02180-02) we have been exploring the possibility that the dynorphin and GAD-immunoreactive fibers in the zona reticulata, have their source in a common, ie. coexisting neuron. Using standard indirect and ABC immunohistochemical techniques, we have been using 5-10 micron brain sections to explore this possibility. Preliminary results suggest that these neuron populations are distinct from one another.

Cholera toxin has been shown to be taken up and anterogradely transported by a subset of dorsal root ganglion cells i.e. large diameter cells innervating the deep dorsal horn and the ventral horn of the spinal cord. It is known that carbonic anhydrase is found primarily in the la afferent neurons. We have been exploring the possibility that the same cells which show cholera toxin immunoreactivity also show carbonic anhydrase immunostain thus possibly providing a specific tracer for this subset of dorsal root ganglion cells. Preliminary studies have revealed that, in fact, the large diameter dorsal root ganglion cells do stain for carbonic anhydrase.

Finally, as part of our interest in the cholinergic projections to the substantia nigra (see MH-02180-02), we developed a technique for identifying cholinergic cell bodies in the brainstem reticular-formation. This method which employs NADPH-diaphorase provided as with a means of confirming cholinergic cell bodies in the pontine reticular formation which have been described as projecting to the nigra. These studies were employed directly in combination with electrophysiological studies of cholinergic activity in the nigra.

Projected Course:

We hope to use our various immunohistochemical techniques to continue our investigations of the hypothalamus. In particular with regard to the presence of coexisting systems, we plan to explore the presence or absence of CRF, CCK and vasopressin immunoreactivity in several physiological states including embryonic, and various stages of estrous and pregnancy. More specifically, the direct

anatomical relationship between the VIP cells of the caudal hypothalamus and the median eminence will be explored. Intraneuronal connections between cells and terminals of different transmitter content will be studied at the level of electron microscopy in the PVN using double and triple immunohistochemistry by means of IgG gold, avidin-ferritin and DAB.

In addition, we plan to make a complete map of the afferents and efferents of the bed nucleus of the stria terminalis preliminary to functional, i.e. electrophysiological studies of this limbic structure. We hope to continue our exploration of the coexistence of dynorphin and GAD as a part of ongoing electrophysiological experiments (see MH-02180-02). Similarly, as studies are relevant to our functional experiments, cholinergic projections to the mesencephalon will be continued. We hope to further define the raphe system projecting to the phrenic motor nucleus to see if it is the tri-transmitter or bi-transmitter system which provides innervation to this respiratory center. Finally, we will continue studies on the co-staining of carbonic anyhydrase and cholera toxin in dorsal root ganglion cells in the search for a specific tracer for Ia afferents.

Significance to Biomedical Research:

In the nervous system, neurons communicate with each other mainly through the use of chemical transmitters. The growing number of putative peptide transmitters localized to specific nuclei in the brain has exponentially increased the complexity of potential transmitter interactions which may define function. The use of immunohistochemical and retrograde tracing techniques provide a means of revealing in detail the exact loci in the brain for specific antigens, ie, putative transmitters. Thus these techniques will provide primary information to the cellular strategies by which cell communicate primary to electrophysiological studies in our laboratory (see MH-02180-02).

In particular, the ACTH response to stress which is thought to be regulated by specific neurons in the hypothalamus is modified in several clinical states. Thus, we hope that our studies on the morphology of these systems will provide new information on the domains over which transmitters operate and thus prove essential to cogent functional studies of the brain.

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5) Hokfelt, T., Skirboll, L., Everitt, B., Meister, M., Brownstein, M., Jacobs, T., Faden, A., Kuga, S., Goldstein, M., Markstein, R., Dockray, G. and Rehfeld, J. Distribution of cholecystokinin-like immunoreactivity in the nervous system with special reference to coexistence with classifical neurotransmitters and other neuropeptides. In Neuronal Cholecystokinin, N.Y. Academy of Sciences, 1985 pp. 255-272.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02177-03 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurobiology of Depression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.N. Crawley	Senior Staff Fellow	NS, NIMH
Others:	L.R. Skirboll	Staff Fellow	NS, NIMH
	D.W. Hommer	Staff Psychiatrist	NS, NIMH
	S.M. Paul	Chief	NS, NIMH
	W. Mendelson	Staff Psychiatrist	CP, NIMH
	J. Martin	Staff Fellow	CP, NIMH

COOPERATING UNITS *(if any)*

Electrophysiology Unit, Section on Molecular Pharmacology, NS, NIMH;
Unit on Sleep Studies, Clinical Psychobiology Branch, NIMH.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The past decade has witnessed the discovery of forty or more peptides localized in neurons of mammalian brain. Many cases of peptides coexisting in the same neuron with classical transmitters have been described. Our laboratory is investigating the functional significance of coexisting peptides and transmitters in the central nervous system, using behavorial tools. Our approach involves cannulating the postsynaptic nucleus containing the nerve terminals and postsynaptic receptors of pathways in which neuropeptides and neurotransmitters coexist. Behavorial actions of the transmitter, the peptide, and combinations of transmitters and peptide(s), microinjected directly into the postsynaptic site, and then evaluated to test for potential interactions between the behavioral effects of the transmitter and the peptide(s).

A) We previously showed that cholecstokinin (CCK) potential dopamine-induced hyperlocomotion in the nucleus accumbens, where CCK and dopamine coexist. This year, antagonists of CCK were analyzed for their pharmacological specificity in blocking the CCK modulation of dopaminergic function. Both microinjections into the nucleus accumbens and intraperitoneal systemic injections of proglumide and benzotript specifically blocked the ability of CCK to potgentiate dopamine-indiced hyperlocomotion in the nucleu accumbens. This finding demonstrates that a clinically useful route of administration of a CCK antagonist can block central CCK function, suggesting that CCK antagonists may be novel antipsychotic agents in reducing dopaminergic function in the mesolimbic pathway.

B) Substance P (SP), corticotropin releasing factor (CRF) and acetylcholinesterase (Ach E) were found to coexist in dorsolateral tegmental neurone projecting to the rat prefrontal cortex. The cholinergic agonist, carbachol, microinjected into the prefrontal cortex, induced a profound stereotyped motor behavior resembling "boxing." SP potentiated carbachol-induced "boxing." The functional significance of this triple coexistence, therefore, may be an upregulation by one peptide, and a down regulation by the other peptide, of the function of the primary transmitter.

OBJECTIVES

A) Analysis of cholecystokinin (CCK) antagonists as pharmacological treatments to block the potentiation of dopamine by CCK in the mesolimbic system.

B) Investigation of the functional significance of a triple coexistence of acetylcholine, substance P, and corticotropin releasing factor in the prefrontal cortex.

METHODS

Aspectic stereotaxic implanation of indwelling cannulae into brain nuclei of rats. Microinjection of neurochemicals into discrete anatomical nuclei. Intraperitoneal injection of drugs. Behavorial analysis of neurochemical effects, including locomotion, seizures, and stereotyped motor behaviors. Histological verification of cannulae placement and injection sites.

MAJOR FINDINGS

A) Central injections of CCK antagonists, directly into the nucleus accumbens, effectively blocked the ability of CCK to potentiate dopamine-induced hyperlocomotion. Proglumide and benzotript completely blocked CCK, but at dose four orders of magnitude above the active CCK dose. Neither antagonist had an effect on locomotion when administered alone, and neither blocked the effects of dopamine on locomotion.

Given this positive data on two CCK antagonists injected centrally, we embarked on a series of studies to test the activity of systemically administered CCK antagonists to block the central action of CCK. These studies were designed evaluate a clinically relevant route of administration of CCK antagonists. Our hypothesis is that a CCK antagonist may act as an antipsychotic treatment, either alone or in combination with a dopamine antagonist. Rationales behind this hypothesis include 1) CCK potentiates dopaminergic function, therefore a CCK antagonist would block an endogenous potentiator of central dopamine systems; since antagonists are not peptides, they cross the blood-brain barrier and are not quickly metabolized, therefore they may be practical for drug therapies.

Proglumide (1 mg/kg, i.p.) effectively blocked the CCK potentiation of DA-induced hyperlocomotion. Benzotript (10 mg/kg, i.p.) effectively blocked the CCK potentiation of DA-induced hyperlocomotion. Neither antagonist affected locomotion when given alone or affected DA-induced hyperlocomotion. Therefore, intraperitoneal injection of a CCK antagonist can effectively and specifically block the ability of CCK to facilitate dopaminergic function in the mesolimbic pathway.

Proposed Course of Project:

A) Our hypothesis that CCK antagonists may be effective antipsychotics rests on the premise that endogenous CCK is released with dopamine in psychosis, and that a CCK antagonist given systematically will block the ability of endogenous

CCK to potentiate dopamine. To date, all studies have administered CCK exogenously into the nucleus accumbens. We have been searching for a method to stimulate release of endogenous mesolimbic CCK without pharmacological intervention. Two behavioral techniques are currently being tested: footshock stress and treadmill running. These two behavioral challenges have been demonstrated to cause increased release of dopamine in the nucleus accumbens. In the nucleus accumbens, dopamine induces hyperlocomotion. Therefore, if CCK is released with dopamine following footshock or treadmill running, then hyperlocomotion should occur following footshock or treadmill running. We will then test the ability of CCK antagonists to block a portion of the hyperlocomotion induced by the footshock or treadmill. If proglumide and/or benzotript reduces hyperlocomotion which is induced behaviorally, then functional role of endogenous CCK in modulating endogenous dopamine may be deduced. Combined doses of haloperidol and proglumide will then be tested for the optimal combination to block the behavioral effects of endogenous overactivity of the mesolimbic pathway.

Significance to Biomedical Research

A) The coexistence of CCK and dopamine in the mesolimbic pathway of rats and human provides a new level of modulation of neurotransmitter function, and new possibilities for therapeutic treatments of neuropsychiatric disorders. Development of antipsychotic treatments which combine low doses of a neuroleptic with a cholecystokinin antagonist would provide a new treatment for schizophrenia with a reduced risk for the development of tardive dyskinesias.

Major Findings

B) Coexistence of substance P (SP), corticotropin releasing factor (CRF) and acetylcholinesterase (AChE) was demonstrated by immunocytochemical staining and retrograde tracing in neurons of the lateral dorsal tegmental nucleus, projecting to the prefrontal cortex, septum and thalamus in rat brain, by Dr. David Jacobowitz, NIMH, IRP. In collaboration with Dave, we proposed to test the functional significance of the triple coexistence of SP, CRF, and acetylcholine

SP, CRF, the cholinergic agonist, carbachol, or saline was microinjected through bilateral indwelling guide cannulae into the medial frontal cortex of awake rats. The first series of behavioral observations included recording of the following behaviors: grooming, sniffing, locomotion, exploration, vertical movements, eyes closed, resting time, resting posture, limb orientation, tail orientation, and forepaw movements. Doses of SP and CRF up to 5 µg did not produce any behavorial activity on any of these parameters over observation periods up to 2 hours after microinjection. Carbachol, 1 µg-6 µg, produced a profound behavioral syndrome, consisting of repetitive forepaw treading while the rat was reared up on his hindlegs in a vertical upright posture. We are calling this motor pattern "boxing", as it resembles the rapid punching movements made by a human boxer.

To functionally characterize "boxing", we discussed the syndrome with experts in the NIMH intramural program in terms of motor patterns elicited from the rat frontal cortex. Dr. Steve Wise examined the histological placement of our

cannula, concluding that the microinjections were limited to cortical layers 4 and 5, regions not known to elicit stereotyped motor patterns. In collaboration with Dr. Wally Mendelson and Dr. Joe Martin, we placed electroencephalographic electrodes in the frontal cortex along with cannulae in the medial frontal cortex. EEG analysis showed high amplitude spiking following microinjection of carbachol 1 µg - 4 µg. Spiking was temporally correlated with observable bouts of "boxing." We therefore suggest that "boxing" is a motor expression of an unusual form of seizures elicited by carbachol in the rat prefrontal cortex.

To pharmacologiclly characterize "boxing," two series of experiments were performed. Pretreatment with anticonvulsant drugs, including clonazepam, diazepam, and pentobarbital, blocked carbachol-induced "boxing." This supports the interpretation of "boxing" as a form of seizures. Pretreatment with the muscarinic antagonist, atropine, blocked carbachol-induced "boxing," while pretreatment with the nicotinic antagonist, mecamylamine, did not block carbachol-induced "boxing." These results suggest that a muscarinic cholinergic receptor mediates "boxing."

The next series of experiments was designed to test the hypothesis that the peptides SP and CRF, which coexist with acetylcholine in the prefrontal cortex, modulate the function of the classical transmitter, acetylcholine. SP or CRF was coinjected with carbachol through the bilateral indwelling cannulae in the medial frontal cortex. SP (1 µg) was found to significantly potentiate carbachol-induced "boxing." CRF (10 ng) was found to significantly inhibit carbachol-induced "boxing." Neither peptide induced "boxing" when injected alone at doses up to 5 µg. Calcitonin gene related peptide (CGRP) is also located in the medial frontal cortex of the rat, but does not coexist with acetylcholine. Coinjection of CGRP with carbachol had no effect on carbachol-induced "boxing" doses of CGRP up to 200 ng. These data suggest that the two peptides which coexist with acetylcholine can modulate its function, while a peptide in the same location but not coexisting cannot. It is tempting to speculate that the functional significance of a triple coexistence involves one peptide up-regulating, and the second peptide down-regulating, the action of the primary transmitter.

Proposed Course of Project:

B. Studies are in progress and planned to investigate the possibility of anatomical specificity of SP and CRF modulation of carbachol-induced "boxing" to areas of SP-CRF AchE coexistence. Studies are also in progress and planned to test the pharmacological specificity of SP and CRF modulation by analyzing other peptide found in the frontal cortex but not coexisting with acetylcholine. Further, peptides and peptide antagonists are being tested for their ability to reverse the seizures induced by carbachol.

Significance to Biomedical Research

B) The coexistence of SP, CRF and AchE in the prefrontal cortex provides a new level of modulation of neurotransmitter function, and new possibilities for therapeutic treatments of seizure disorders. Our very preliminary data shows that a substance P antagonist can block carbachol-induced "boxing." This early finding

points to the possibility that a SP antagonist might be a new candidate for an antiepileptic drug therapy.

Publications:

1. Crawley, J.N., Hommer, D.W., and Skirboll, L.R. Behavioral and neurophysiological evidence for a facilitory interaction between co-existing transmitters: cholecystokinin and dopamine. Neurochemistry International 6:755-760, 1984.

2. Crawley, J.N. Potentiation of dopamine-mediated behaviors by CCK in the nucleus accumbens. Annals of the New York Academy of Sciences, "Neuronal Cholecstokinin", edited by J.J. Vanderhaeghen and J.N. Crawley, 1985.

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9. Crawley, J.N., Clarification of the behavioral functions of peripheral and central cholecystokinin: two separate pools. Peptides, in press.

10. Crawley, J.N., Stivers, J.A., Hommer, D.W., Skirboll, L.R., and Paul, S.M. Antagonists of central and peripheral behavioral actions of cholecystokinin J. of Pharmacol. and Exp. Ther., in press.

11. Crawley, J.N., Olschowka, J.A., Diz, D.I., and Jacobowitz, D.M. Behavioral investigation of the coexistence of substance P, corticotropin releasing factor, and acetylcholinesterase in lateral dorsal tegmental neurons projecting to the medial frontal cortex of the rats. Peptides, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02178-03 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropharmacology of Anxiety

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.N. Crawley	Senior Staff Fellow	NS, NIMH
	R.C. Drugan	Postdoctoral Fellow	NS, NIHM
Others:	L.R. Skirboll	Staff Fellow	NS, NIMH
	D.W. Hommer	Staff Psychiatrist	NS, NIMH
	S.M. Paul	Chief	NS, NIMH
	P. Skolnick	Pharmacologist	LBC, NIADDK

COOPERATING UNITS *(if any)*

Electrophysiology Unit, Section on Molecular Pharmacology, NS, NIMH; Unit on Sleep Studies, Clinical Psychobiology Branch, NIMH, Laboratory on Bioorganic Chemistry, NIADDK.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Animal models of anxiety are being used to investigate the biological mechanisms underlying anxiety-related behaviors. Anatomical sites mediating responses to benzodiazepines and GABAergic drugs are being tested, using the thirsty-lick conflict model. Muscimol, 5 µg, produced a significant anxiolytic response when microinjected into the lateral septum. Neurophysiological sensitivity to muscimol was increased in substantia nigra neurons after tailshock stress. Inescapable tailshock stress also decreased the number of ^{3}H-Ro5-4864 binding sites in rat cerebral cortex, heart and kidney.

OBJECTIVES:

The recent discovery of potent ligands for the brain benzodiazepine binding site has led to intensive investigation of anxiogenic and anxiolytic properties of the brain benzodiazepine-GABA-chloride ionophore receptor complex. We are investigating several aspects of the benzodiazepine receptor in terms of its role in anxiety-related behaviors:

1) Anatomical pathways mediating anxiety-related behaviors;

2) Ability of stress-inducing stimuli to change the number of benzodiazepine binding sites;

3) Ability of stress-inducing stimuli to change the electrophysiological sensitivity to muscimol;

4) Relationship between anxiety-related behaviors and depression related behaviors.

Methods Employed:

Rat thirsty-lick conflict test, rat learned helplessness test, benzodiazepine binding assays, extracellular neurophysiological recording, intracerebral cannulation and microinjection, histological verification of injection sites.

Major Findings:

Dr. Robert Drugan, a postdoctoral fellow (NRSA award) in our unit, has brought the learned helplessness model of depression from the laboratory of Dr. Steve Maier, University of Colorado, to our branch. Using our cannula implantation and microinjection techniques, Rob has found that the GABA agonist, muscimol, has an anxiolytic action when injected into the lateral septum of rats. The dose-response curve for this effect is steep, with sedation beginning at doses less than three times the anxiolytic dose. The observed narrow window for muscimo may explain lack of evidence in the literature for anxiolytic actions of GABAergic drugs. Benzodiazepines may act by fine-tuning the relatively all-or-none response of the inhibitory GABA system.

In collaboration with Dr. Daniel Hommer, Dr. Drugan has analyzed the electrophysiological response to muscimol in substantia nigra cell bodies in rats treated with inescapable tailshock. The behaviorally stressed animals showed an increased sensitivity to muscimol as compared to unstressed animals. To our knowledge, this is the first report of a behavioral stressor inducing a shift to the left of the dose-response curve for a GABAergic agonist in an acute electrophysiological preparation.

We are also collaborating with Phil Skolnick, LBC, NIADDKD, in studies of the peripheral-type benzodiazepine binding site. After inescapable footshock,

binding is significantly decreased in the kidney, heart, and cerebal cortex. This decrease in binding sites suggests that the peripheral-type benzodiazepine receptor may also be under neural control, e.g., via the sympathetic nervous system, and regulated by environmental stressors.

Proposed Course of the Project:

The lateral septum site will be further investigated for its role in anxiety-related behaviors. Benzodiazepine agonists and antagonists will be tested for their actions as anxiolytics and anxiogenics when injected into the lateral septum, using the thirsty-lick conflict test. In addition, the lateral septum site will be tested with the same drugs on the learned helplessness model of depression, to test the hypothesis that the development of the learned helplessness syndrome is partially a function of anxiety generated by inescapable tailshock.

Significance to Biomedical Research:

The immediate goal of this series of experiments is a complete description of anatomical sites and pathways integrating anxiety-related behaviors in rats. The ultimate goal is an understanding of the neuroanatomical and pharmacological dysfunctions underlying human anxiety. In addition, the possible neuroanatomical and neuropharmacological relationships between anxiety and depression which will be tested may result in psychopharmacological treatments specific for the anxious or agitated subtype of human depression.

Publications:

1. Crawley, J.N., Ninan, P.T., Pickar, D., Chrousos, G.P., Linnoila, M., Skolnick, P. and Paul, S.M. Neuropharmacological antagonism of the β-carboline-induced "anxiety" response in rhesus monkeys. J. of Neurosci. 5: 477-485, 1985.

2. Crawley, J.N. Exploratory behavior models of anxiety in mice. Neurosci. Biobehav. Rev. 9: 37-44, 1985.

3. Skolnick, P., Ninan, P., Insel, T., Crawley, J., and Paul, S. A novel chemically induced animal model of human anxiety. Psychopathology 17: suppl. 1, 26-36, 1984.

4. Skolnick, P., Ninan, P., Insel, T., Crawley, J., and Paul, S. Benzodiazepine receptor-mediated "anxiety" in primates. Psychiatry vol. 2 ed. P. Pichot, P. Berner, R. Wolf, and K. Thau, Plenum Press, NY, 1985.

5. Drugan, R.C., Maier, S.F., Skolnick, P., Paul, S.M., and Crawley, J.N. A benzodiazepine receptor antagonist induces learned helplessness. Eur. J. Pharmacol. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02179-03 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hamster Separation Model of Depression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	S.M. Paul	Chief	NS, NIHM
	P. MacLean	Chief, LBEB, NIMH	LBEB, NIMH
	T. Insel	Psychiatrist	CN, NIMH
	C. Pert	Chief, Section on Brain Biochemistry	NS, NIMH
	M. Linnoila	Clinical Director	ALC, NIAAA

COOPERATING UNITS *(if any)*

Laboratory of Brain Evolution and Behavior, NIMH; Alcohol Intramural Research Program, National Institute of Alcohol Abuse and Alcoholism

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A novel hamster separation model of depression is under development for analysis of central neurochemical changes during a behavior state of separation which may be analogous to human depression. Separated male dwarf hamsters show increased body weight, decreased exploratory behaviors, and reduced social interactions. High pressure liquid chromatography assays of brain monoamines and metabolites failed to detect significant differences in separated hamsters as compared to hamsters remaining in male-female pairs. Autoradiographic assays of opiate receptors failed to detect significant differences between separated and paired hamsters on 3H-naloxone binding.

OBJECTIVES

Development and evaluation of a new rodent model for depression. Characterization of specific, reproducible, quantitative changes in behavior. Evaluation of pharmacological specificity of antidepressant treatments in reversing the behavioral changes. Investigation of the changes in brain receptor sensitivity and neurotransmitter function during the defined state of social separation in the Siberian dwarf hamster.

Methods:

Breeding, pairing, and separating of Siberian dwarf hamsters, behavioral rating of exploratory and social behaviors; rating of body weight, high pressure liquid chromatography analysis of brain monoamines and metabolites.

Major Findings:

Early pilot studies had shown a significant reduction in serotonergic activity, as measured by HPLC assay of 5-HIAA/5-HT ratios in the cerebral cortex and diencephalon of separated as compared to paired male dwarf hamsters. In collaboration with Dr. Markku Linnoila, a larger, more complete study was undertaken to assay 5-HT, 5-HIAA, DA, DOPAC, HVA, NE, and MHPG in cerebral cortex, diencephalon, and mesecephalon of separated and paired male dwarf hamsters. No significant differences were obtained between separated and paired hamsters at the $P<.05$ level of significance in any brain region on any transmitter or metabolite. A trend was seen for a reduced 5-HIAA/5-HT ratio, as previously found. The hypothesized serotonergic site for the separation syndrome remains in question. Imipramine was found to partially reverse the syndrome. Twelve new pairs of hamsters have been bred, pretested, and 6 of the pairs separated, for a new drug trial with desmethylimipramine. If DMI is as effective or more effective than imipramine, then the serotonin system may not be the critical site mediating the hamster separation syndrome.

In collaboration with Tom Insel and Candace Pert, we have tested the hypothesis that opiate receptors would be changed in separated male dwarf hamsters. We bred, pretested, separated, and tested hamsters for autoradiographic analysis of ^{3}H-naloxone binding. Dr. Insel could not detect any significant differences in binding in separated as compared to paired hamsters, although quantitation of binding in discrete anatomical sites has not been completed.

Proposed Course of the Project:

The next experiment will test the ability of the tricyclic antidepressant, desmethylimipramine, which blocks norepinephrine uptake, to reverse the separation syndrome. Continuing drug trials are designed to test specificity of the syndrome to the antidepressant category of drugs. Continuing assays of brain neurotransmitters, neuropeptides, and their receptors are designed to reveal neuroanatomical and pharmacological mechanisms mediating the separation syndrome.

Significance to Biomedical Research and the Program of the Institute:

Phodopus sungorus is a rapidly breeding, easily maintained species. Its usefulness as a model for human depression will be determined over the next two years. Such a model could be applied to the development of new classes of antidepressants. At the level of basic research, the model could provide a discrete population for testing current theories of neurochemical dysfunctions in depression.

Publications:

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2. Crawley, J.N. A monoamine oxidase inhibitor reverses the "separation syndrome" in a new hamster separation model of depression. *European Journal of Pharmacology*, in press.

3. Crawley, J.N., Sutton, M.E., and Pickar, D. Animal models of self-destructive behavior and suicide. *Psychiatric Clinics of North America*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02180-03 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Electrophysiological Studies of Peptidergic and GABAergic Function in Mammalian Brain.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Hommer	Staff Psychiatrist	NS, NIMH
	L. Skirboll	Staff Pharmacologist	NS, NIMH
Others:	J.N. Crawley	Pharmacologist	NS, NIMH
	S.M. Paul	Chief	NS, NIMH
	B. Robertson	Guest Researcher	NS, NIMH
	G. Stoner	Guest Researcher	NS, NIMH
	M. Palkovits	Visiting Scientist	LCB,NIMH
	P. Clarke	Fogarty Fellow	BP, NIMH

COOPERATING UNITS (if any)

Laboratory of Cell Biology, NIMH;
Biological Psychiatry Branch, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using extracellular single unit recording techniques, we have examined the effects of stress and pharmacological agents which either alleviate or mimic the effects of stress on individual neurons in the rat substantia nigra (SN). Specifically, learned helplessness induced by uncontrollable stressful shocks results in a supersensitivity to gamma-amino butyric acid (GABA) agonists while shocks which are controllable do not produce GABAergic supersensitivity. The anxiogenic benzodiazepine (BZ) receptor ligand, beta-carboline carboxylate ethyl ester (βCCE) increases the activity of neurons in the SN zona reticulata (ZR) but had no effect on noradrenergic neurons in the locus coeruleus. Caffeine also mimics many of the effects of βCCE in the SN but its actions are not reversed by the specific BZ antagonist Ro-15-1788 as are those of βCCE. Furthermore, the recently isolated putative endogenous peptide ligand for the BZ receptor, diazepam binding inhibitor (DBI), excites ZR neurons. These effects could not be reversed by Ro-15-1788 suggesting that this peptide may not act through the BZ receptor.

We have continued our studies on the interactions between endogenously occurring neuropeptides and classical neurotransmitters. All varieties of CCK-like peptides which bind to brain CCK receptors also potentiate DA in those areas where CCK and DA coexist while those CCK-like peptides which do not bind to this receptor are ineffectual in facilitating DA inhibition. The putative cholecystokinin (CCK) antagonists, proglumide and benzotript, were found to weakly block CCK in proportion to their potency at central CCK receptors.

Dynorphin (DYN) appears to modulate the response of SNZR neurons to GABA. Finally, we have examined the effects to cholinergic agents in the SN. Nicotinic agents appear to activate DA neurons in the SN through a central mechanism.

PROJECT DESCRIPTION

OBJECTIVES

The SNZR is a region of the brain which contains a high concentration of GABA, BZ receptors as well the opiate-like peptide, DYN. Recently several groups have shown that microinjections of GABA agonists into the SN, but not into adjacent mesencephalic areas, blocks electrically-induced seizures in rats. These microinjections also block the limbic afterdischarge following kindled seizures. This suggests that the SN may be an important region involved in the propagation of seizure activity as well as in modulation of limbic system function. Since BZ can block seizures and alter behavior presumably mediated through limbic regions, the SNZR represents an ideal location in which to study BZ actions using electrophysiological techniques. Furthermore, since GABA, BZ's and opiates all have been implicated in the neural response to stress, the SN represents an excellent system in which to study the effects of stress as well as the drugs and putative transmitters which may modulate stress response.

Aside from peptides and GABA present in the SN, it has also been reported that the SN is an area in nicotinic receptors. In this regard, we have been examining the cholinergic influence on the activity of nigral neurons. We have explored the effects of nicotine on the firing of neurons in the SNZR and SNZC neurons.

Immunohistochemical studies have shown that there is a coexistence of DA and CCK in a subpopulation of mesencephalic neurons in the rat which project primarily to limbic areas. We have previously reported that these DA-CCK containing cells are excited by either systemically or iontophoretically administered CCK, we have investigated the question of the functional significance of this coexistence (i.e. how does the peptide CCK and the classical neurotransmitter, DA, interact to affect the activity of neurons). In addition, we have begun to investigate the interaction between other classical neurotransmitters (GABA) and peptides (DYN) in the SNZR. These experiments provide another avenue toward developing an understanding of the functional relationship between neurotransmitter systems.

METHODS EMPLOYED

(SEE: 1984 Annual Report, pp. 767-771, Project Number Z01 MH 02180-02 NS Electrophysiological Studies of Peptidergic and GABAergic Function in Mammalian Brain.)

MAJOR FINDINGS

1. Effects of stress on GABAergic sensitivity in the SNZR.

When rats are subjected to tail shock which they cannot control, this experience produces a supersensitive response to the intravenously administered GABA agonist, muscimol. This effect appears to related to the uncontrollable nature of the shock. Animals which are subjected to an identical shock exposure but over which they have some control do not develop a supersensitive response to muscimol.

Thus, uncontrollable stress leads to an increased sensitivity of SNZR neurons to the inhibitory actions of GABAergic agents.

2. Effects of drugs which may mimic the effects of stress.

Caffeine decreases the activity of DA neurons in the ventral tegmental area (VTA) which project to limbic regions but has little effect on DA neurons in the more later SN regions which project to the striatum. This caffeine-induced inhibition can be blocked by either diazepam or haloperidol suggesting that both the BZ receptor and DA release may play a role in mediating caffeine's actions.

3. CCK-DA interactions

As we have previously reported, ceruletide and sulfated CCK-8 (CCK-8-US) both potentiate the effects of the DA agonist, apomorphine, on DA neurons in the medial SN. We have also found that unsulfated CCK-8 (CCK-8-US) and CCK-4 possess a similar ability to potentiate apomorphine induced inhibition in the SN. The rank order potencies were as follows: ceruletide > CCK-8-S = CCK-8-US > CCK-4; CCK-3 was without effect. These potencies directly parallel the affinity of these peptides for the brain CCK receptor. In contrast, only CCK-8-S produced an excitatory effect on DA neurons while CCK-US, CCK-4 and CCK-3 were devoid of effect. This profile of activity corresponds to the affinities of the various CCK-like peptides at the peripheral CCK binding site. It appears that CCK's different CNS effects may be mediated by different CCK receptors.

4. GABA-DYN interactions.

We have found that DYN, an opiate-like peptide present in high concentrations in the SNZR modulates the effects of the neuronal inhibition produced by GABA applied directly to nigral neurons. DYN had two distinct actions; either potentiation or attenuation of the effects of GABA. In cells which were themselves inhibited by DYN, the peptide potentiated GABA's actions. In those cells which were unresponsive to DYN, simultaneous application of DYN and GABA resulted in an attenuation of the GABA inhibition. These findings provide further evidence for the modulatory interaction between peptides and classical neurotransmitters.

5. Nicotinic influences on nigral activity.

Low systemic doses of nicotine stimulated the firing of SNZC DA cells; experiments with antagonists showed this action to be due to a central and probably direct action of nicotine on these cells. Nicotine also excited non-DA cells in the SNZR, but this action was clearly of peripheral origin. These results are in accord with autoradiographic data showing that nicotinic binding sites in the SN are largely restricted to the SNZC.

PROJECTED COURSE

We plan to continue our investigations of the effects of stress in the SN and expand our efforts to include examinations of other neurotransmitters such as DA and enkephalins as well as GABA. We also plan to more fully characterize the

phenomenon of increased sensitivity to GABAergic agents through the use of iontophoretic techniques and an examination of the role of stress related hormones such as the corticosteroids.

Studies of the interactions between classical transmitters and peptides will be extended along several lines. First, the modulation of the GABA response by DYN will be compared to other peptide like Substance P and the enkephalins. Secondly, the hypothalamus will be explored as a site of multiple transmitter interaction. In immunohistochemical studies (see Z01 MH 00179-04 NS), we have been exploring the transmitter systems which innervate the paraventricular nucleus. These transmitters can be altered by adrenalectomy or systemic administration of a phenyl-n-methyl-transferase inhibitor. We plan to explore the functional correlate of these findings. That is, look at the electrophysiologic response of paraventricular nucleus neurons to neuropeptide Y, adrenaline and adrenalectomy.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

Studies of the interaction between transmitters (CCK-DA and GABA-DYN) may provide a prototype for modulatory function between peptide and classical transmitters. Furthermore, electrophysiological studies of BZ and GABA complement ongoing biochemical and behavioral studies in these areas. The SN appears to be an important brain region in the modulation of seizures. Electrophysiological techniques are particularly well suited to further our understanding of how the SN performs this function. In addition, the hypothalamus is the site of control of many vegetative and neuroendocrine functions which control response to stress. Thus, understanding how stress affects the nigral and hypothalamic systems may be of potential value in developing pharmacological approaches to stress related diseases, both medical and psychiatric.

Finally, investigation into the actions of nicotine on the DA system may further our understanding of the reinforcing properties of the nicotine contained in tobacco. Thus, data on this system may increase our knowledge of the biological basis of the nation's most widespread addiction.

PUBLICATIONS

Weber, K.H., Kuhn, E.J., Boke-Kuhn, K., Lehr. E., Danneberg, P.B., Hommer, D.W., Paul, S.M., and Skolnick, P.: Pharmacological and neurochemical properties of 1,4-diazepines with two annelated heterocycles ("hetrazepines"). Eur. J. Pharmacol., 109: 19-31, 1985.

Skirboll, L.R., and Hommer, D.W.: Electrophysiological Studies of the Role of Cholecystokinin in the Substantia Nigra and Its Interactions with Dopamine. Ann. N. Y. Acad. Sci. 448: 275-282, 1985.

Crawley, J.N., Hommer, D.W., Skirboll, L.R.: Behavioral and neurophysiological evidence for a facilatory interaction between co-existing transmitters: cholecystokinin and dopamine. Neurochem. Int. 6: 755-760, 1984.

Hommer, D.W., M. Palkovits, J.N. Crawley, S.M. Paul, and L.R. Skirboll. CCK-induced excitation in the substantia nigra: evidence for peripheral and central components. J. Neurosci., 5: 1387-1392, 1985

Crawley, J.N., D.W. Hommer, and L.R. Skirboll. Topographical analysis of nucleus accumbens sites at which cholecystokinin potentiates dopamine-induced hyperlocomotion in the rat. Brain Res., 355: 337-341, 1985.

Hommer, D.W. and S.M. Paul. Benzodiazepines, neurotransmitters and the neurobiology of anxiety. In Giannini, J., Gold, M. and Extein. I. (Eds.): Introduction to Biological Psychiatry, in press.

Clark, P.B.S., A.Pert, D.W. Hommer, and L.R. Skirboll. Electrophysiological actions of nicotine on substantia nigra single units. Br. J. Pharmacol., in press.

Crawley, J.N., J.A. Stivers, D.Hommer, L. Skirboll, and S.M. Paul. Antagonists of central and peripheral behavioral actions of cholecystokinin. J. Pharmacol. Exp. Ther., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02182-03 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Toward the Visualization of Opiate Receptors in Living Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. B. Pert	Pharmacologist	NS, NIMH
Others:	M. A. Channing	Physician	NM, CC
	W. C. Eckelman	Chief, Rad. Chem. Sec.	NM, CC
	S. M. Larson	Chief	NM, CC
	T. R. Burke, Jr.	Pharmacologist	LC, NIADDK
	K. C. Rice	Pharmacologist	LC, NIADDK

COOPERATING UNITS *(if any)*

Nuclear Medicine, Clinical Center and Laboratory of Chemistry, NIADDK

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The distribution of positron emitting substances in brain can be followed by positron emission tomography (PET). We are developing ^{18}F-labeled high affinity opiate drugs to be injected into living humans for the visualization of opiate receptor patterns in vivo. It will be interesting to determine whether opiate receptor distribution patterns in cortex change as a function of attention and emotional states.

Project Description:

Objectives:

To demonstrate gradients of opiate receptor density in the cortex of living humans. To examine whether differences in these gradients exist as a function of emotional state or attentional processes.

Methods Employed:

PET Scan--using newly developed ^{18}F-labeled opiate analogs. Autoradiography of rat brain slices.

Major Findings:

We managed to affix a fluoride moiety to naltrexone, a potent opiate antagonist without losing affinity for opiate receptors. This fluoro-opiate derivative is suitable for in vivo injections for visualizing receptors. We have visualized stereospecific, striking images for opiate receptors in the thalamus, basal ganglia and frontal cortex of a living baboon. Tritium labeled cyclofoxy has been prepared and shown in rats to have the appropriate opiate distribution pattern.

Significance to Biomedical Research and Program of the Institute:

The notion that alterations in mood are a function of oscillations in neurotransmitter receptor sensitivity is perhaps the most exciting new lead in attempting to understand the causes of mental illness. Other leads in this institute point to the relevance of cortical participation as a critical factor in psychiatric disease. The opiate receptor is the most well-studied of brain receptors and appears to be associated with the pleasure of fulfilled appetite.

Proposed Course:

Our new useful probe, [^{3}H]-3-acetylcyclofoxy, will be characterized thoroughly in rodents to fulfill requirements for human use. Human studies, first on normal controls, then psychiatric patients, should enable a rigorous test of theories of emotions which emphasize neuropeptide receptors.

Publications:

1. Rice, K.C., Konicki, P.E., Quirion, R., Burke, T.R. and Pert, C.B. Synthesis and pharmacological characterization of (±)-5,9 alpha-dimethyl-2-[2(4-fluoro-phenyl)ethyl]-2'-hydroxy-6,7 benzomorphan (Fluorophen). A ligand suitable for visualization of opiate receptors in vivo. J. of Med. Chem., 1643-1645, 1983.

2. Burke, T.R., Rice, K.C. and Pert, C.B. Synthesis of 17-methyl and 17-cyclo-propylmethyl-3,14-dihydroxy-4,5α-epoxy-6β-fluoromorphinans (foxy and cyclofoxy) as models of opioid ligands suitable for positron emission transaxial tomography. Heterocycles 23: 99-106, 1985.

3. Pert, C.B., Danks, J.A., Channing, M.A., Eckelman, W.C., Larson, S.M., Bennett, J.M., Burke, T.R. and Rice, K.C. [^{18}F]-3-Acetylcyclofoxy: A useful probe for the visualization of opiate receptors in living animals. FEBS Lett. 177: 281-286, 1984.

4. Rothman, R.B., Danks, J.A., Jacobson, A.E., Burke, Jr., T.R., Rice, K.C. and Pert, C.B. Tritiated-6-beta-fluoro-6-desoxy-oxymorphone: A highly selective ligand for the opiate mu receptor whose binding is characterized by low non-specific binding. Neuropeptides 4: 311-317, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02183-03 NS

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Is Schizophrenia an Autoimmune Neuropeptide Receptor Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. B. Pert	Pharmacologist	NS, NIMH
Others:	R. J. Weber	Senior Staff Fellow	NS, NIMH
	J. G. Knight	Guest Researcher	NS, NIMH
	L. E. DeLisi	Psychiatrist	CHG, NIMH

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, NIMH

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Section on Brain Biochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The notion that schizophrenia has an important autoimmune component has been around for several decades, but has not previously been subjected to analysis by the most sensitive, modern techniques. We have developed a simple sensitive assay for detecting antibodies directed against human brain found in sera of schizophrenic patients and controls. We are now exploring the frequency of these antibodies in schizophrenics vs. controls and characterizing the molecular properties of the brain antigens involved and their distribution by visualization in rodent and human brain.

Project Description:

Objectives:

To develop a simple assay for demonstrating brain-directed autoantibodies in schizophrenic sera; to demonstrate the molecular properties of these brain antigens and their distribution in brain tissue; and to explore the possibility that the antigens are cell surface neuropeptide receptors which mediate the biochemistry of emotion.

Methods Employed:

A novel filtration and centrifugation assay for detecting brain antigens in sera and the (McLean, et al., Brain Res. 278: 255-257, 1983) method for visualizing antibody distribution patterns in brain.

Major Findings:

In collaboration with Dr. Weber, over twenty experiments were performed for the purpose of optimizing the conditions of the antibody detection assay. In an early blind experiment, six of the Clinical Center 4-East ward acute schizophrenics' and controls' sera were examined. The two highest numbers in the assay belonged to the two sickest patients. We utilized the sera from these two patients vs. two controls in every experiment as we worked on optimization. The assay appears to sensitively and repeatedly demonstrate differences between controls and sera from other patients which were screened by Dr. DeLisi. The new patient's serum level appeared elevated even after repeated blood sample withdrawals over a period of one year. We know history of this area and are proceeding caustiously.

A significant (14%) percentage of hospitalized chronic patients have antibrain antibodies titers outside the control range.

A pilot study suggests macrophage chemotaxis is altered in schizophrenics.

Significance to Biomedical Research and Program of the Institute:

Schizophrenia is a crippling psychiatric disease which affects one percent of the general population. A complete, convincing understanding of its etiology would almost certainly lead to better therapeutic strategies and would place this psychiatric illness in a more "normal" context with other diseases of the body.

Proposed Course:

We must now collect a large number of determinations on many sera to describe the incidence in normal and schizophrenic sera as well as correlating psychotic symptoms with antibody titers over time within one patient. We plan to perform appropriate controls for neuroleptic drug treatment. We plan future experiments to test whether the incidence of antibodies directed against brain are much higher in schizophrenics--and perhaps certain subtypes--than in normal controls,

and that this incidence is not due to neuroleptic drug treatment. If schiophrenia is indeed a virally-triggered autoimmune disease, we will prove it and provide replicatable methods for others.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02189-02 NS

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptides and their Receptors are Shared by the Brain and the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. B. Pert	Pharmacologist	NS, NIMH
Others:	R. J. Weber	Senior Staff Fellow	NS, NIMH
	J. M. Hill	Visiting Associate	NS, NIMH
	B. Zipser	Guest Researcher	NS, NIMH
	M. R. Ruff	Immunologist	LM, NIDR

COOPERATING UNITS (if any)

Laboratory of Microbiology and Immunology, NIDR

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neuropeptides, small signal peptides largely known for their role as transmitters of nerve impulses in the brain which mediate mood and emotion, have now been shown to regulate immune system function. Our work reveals that human monocytes have receptors and will respond chemotactically to numerous neuropeptides. Neuropeptides which we have reported on include β-endorphin and other opiates, substance P and bombesin. We have shown that a major class of psychoactive drugs, the benzodiazepines, are also potent chemoattractants. In this case we have directly demonstrated the presence of chemotactic receptors through ligand binding experiments. The presence of diverse, distinct neuropeptide chemotactic receptors on monocytes and other immune system cells suggests the existence of a neuroendocrine link between the brain and the immune system whose purpose is to integrate behavioral and emotional responses with immune system function.

In addition to the presence of neuropeptide receptors we have also been able to demonstrate that human alveolar macrophages store and secrete the neuropeptide bombesin. Neuropeptide synthesis is, therefore, a general feature of various immune cell populations. Such results are consistent with a multi-directional communication network via neuropeptides and their receptors. The purpose of this network is to link the body's cellular defense and repair systems with the nervous and endocrine systems and thereby integrate the internal millieu of the whole organism. The flow of information in this network is perceived by the human organisms emotional and/or altered states of consciousness. Ultimately, this results in behavioral decisions at the whole organism level. Additional work has suggested that a major cause of human cancer, small cell lung carcinoma, may not, as previously thought, arise from lung epithelium but originates from hemopoietic cells when the normal macrophage mediated repair of lung tissue is deranged by continuous heavy smoking.

Project Description:

Objectives:

Neuropeptide effects on immune function: All considerations of health and well-being must necessarily attempt to understand immune system function within the context of the whole organism. For this reason we have considered the role of a class of molecules termed neuropeptides - short chains of amino acids primarily known for their role in nervous system function. What is becoming abundantly clear however is that these compounds, far from acting solely within the confines of the brain, are ubiquitous and have pleiotropic effects, functioning as transmitters, growth hormones, and signal agents for all the body systems. We feel that the neuropeptides are key components of a network whose purpose is to integrate behavior and brain function with immunological and endocrine systems.

In order to establish these points we have decided initially to examine the effects of neuropeptides in several ongoing model systems which deal with the immunological aspects of inflammatory responses, specifically the macrophage/granulocyte component. Macrophages figure prominently in many inflammatory processes, such as arthritis or gingivitis. Inflammation is known to have a neurogenic component, and neuropeptides (such as substance P), released from local nerves, appears to play a role in some inflammatory vascular reactions, as well as in arthritis. We questioned whether such locally released neuropeptides could exert some of their effects through immune cells such as the macrophage.

Our first objective was, therefore, to demonstrate a direct role for select neuropeptides in immune function. Additionally, we wished to consider the possibility that neuropeptides, as a general class of compounds, might have effects on immune function. Sporadic reports of neuropeptide effects on *in vitro* mast cell and lymphocyte function have been made over the last 10 years, however, no unifying concepts from this diverse (sparse) literature have emerged.

Small cell lung cancer as a disease of macrophages: Lung cancer is the leading cause of cancer death in the United States and greater than 25,000 people die each year of a subtype of lung cancer known as small cell (oat cell) lung cancer (SCLC). We have recently proposed that SCLC arises not from lung epithelium but rather from the macrophages present in the lungs of chronic smokers. Our interpretation of the etiology of SCLC emphasizes lung emplysema, inflammation, and tissue damage as a stimulus for myelopoiesis and recruitment of bone marrow derived macrophages into diseased lung. These cells then becomes transformed and give rise to the disease known as SCLC.

Our program is directed toward exploring similarities between SCLC cells and macrophages with the intent to develop novel therapeutic modalities. These studies will also focus on new aspects of inflammatory cell biology particularly the conditions which may lead to cell transformation and progression towards overt neoplastic disease.

Methods Employed:

Currently, we are testing various neuropeptides in several standard assays of marcrophage function. This primarily involves an evaluation of the ability of the peptides to induce directed migration of macrophages and other cell types in Boyden chamber type assays. Many of the known chemotactic agents have additional effects on cell physiology and as active chemotactic peptides are identified they can be evaluated for other actions.

In addition to the functional assay of chemotaxis we have developed radioreceptor binding assays for one of the chemotactically active ligands we have studied (benzodiazepines) and are developing assays for others. These studies will permit a biochemical identification and, eventually, characterization of the receptors for some of these compounds. To date no neuropeptide receptors have been isolated in any system and binding studies (in some cases without any ascribed function) are only now being attempted on immune cells. In concert with these binding studies we are also attempting a preliminary biochemical charac- terization of one of the neuropeptide receptors we have identified on human monocytes (opiate receptor) through the method of chemical cross-linking of a radiolabeled ligand to its receptor.

We are examining immune cells for their possible content of several neuropeptides. Cell extracts are resolved by HPLC fractionation and peptides identified by radioimmunoassay. Specific anti-peptide antibodies are being used as histochemical probes to detect neuropeptides in specific immune cells and tissues.

Major Findings:

The opiate peptides (e.g., enkephalin, β-endorphin) are potent chemoattractants for human monocytes. Pharmacologic specificity can be demonstrated through the use of various agonists and antagonists of the opiate receptor. These compounds are exceeding potent; activity can be detected at 10^{-14} concentrations. Several other neuropeptides have been found to have chemotactic activity for human monocytes with similarly low active concentrations. These include the hypotensive, bradykinin like peptide substance P, and the hypothalamic peptide bombesin. Pharmacologic studies using closely related analogs of these peptides indicate that chemotactic effects are mediated by specific neuropeptide receptors.

The benzodiazepine (e.g., valium) class of drugs are also potent chemoattractants for human monocytes. Binding studies confirm the presence of receptors with the appropriate structure/function relationship. Benzodiazepines are among the most widely prescribed drugs in the USA and no effects on macrophages or immune function have previously been described. The endogenous ligand for this receptor is a neuropeptide, only recently identified.

In addition to studies which have revealed a role for neuropeptides in monocyte chemotaxis we have also been able to show that other cells also express highly specific chemotactic receptors for these compounds. Thus, tumor cells, which may also express migratory potential, will chemotax in response to selected

neuropeptides. This response is not characteristic of all tumor cells but shows selectivity, both with respect to cells which respond and to peptides which are active. We have reported on the ability of neuropeptides such as bombesin, ß-endorphin, substance P, and other to promote SCLC chemotaxis. Ongoing studies reveal that other highly metostatic tumors (e.g., breast) will also respond chemotactically to select neuropeptides. We have also shown that human spermatozoa will migrate chemotactically to various peptides. This new methodology, modified from our monocyte techniques, can now be utilized by researchers in human fertility who have to date not been able to assess sperm motility in any sensitive, quantitative fashion.

Small cell lung cancer: The cell of origin for SCLC has been speculative for 50 years but has focused on a neuropeptide secreting cell sparsely distributed in lung epithelium. Primarily, this is because SCLC cells synthesize a number of neuropeptides; most consistantly bombesin. Our recognition of the importance of macrophages in inflammatory diseases and our studies on neuropeptide synthesis by macrophages prompted a direct test of the hypothesis that the putative neuropeptide secreting precursor was not a lung epithelial cell but another other cell which figured prominently in the pathology of smokers lung, the macrophage. We were able to demonstrate four surface antigens, found only on macrophages and their precursors, to be present on cell lines and tumors of SCLC. These results are now confirmed and extended by other groups and we have interpreted these results to support our suggestion of a macrophage derivation for SCLC.

With regard to our studies on lung cancer it should be noted that no effective therapy exists for this disease and it is rapidly lethal; average life expectance for untreated SCLC is 5-8 weeks. Oral cancer, although less common than lung cancer, is also associated with the use of tobacco products, and the etiology we propose for lung cancer is quite relevant to this disease as well. OUr results have suggested a novel interpretation of the etiology of SCLC which suggests a number of unexplored therapeutic strategems.

The cell of origin for SCLC has been speculative for 50 years but has focused on a neuropeptide secreting cell sparsely distributed in lung epithelium. Primarily, this is because SCLC cells synthesize a number of neuropeptides; most consistantly bombesin. Our recognition of the importance of macrophages in inflammatory diseases and our studies on neuropeptide synthesis by macrophages prompted a direct test of the hypothesis that the putative neuropeptide secreting precursor was not a lung epithelial cell but another cell which figured prominently in the pathology of smokers lung, the macrophage. We were able to demonstrate four surface antigens, found only on macrophages and their precursors, to be present on cell lines and tumors of SCLC. These results are now confirmed and extended by other groups and we have interpreted these results to support our suggestion of a macrophage derivation for SCLC.

Among the growth regulating hormones which control monocyte growth and differentiation are the interferons and colony stimulating factors. These and other immune hormones can now be evaluated systematically within this new conceptual framework for their effect on SCLC cells. The recent cloning of both of these hormones makes this an attractive approach as ample precident documents the

ability of these agents to modify leukemia cell growth in some settings. Various combined modalities may ultimately prove more efficacious than current treatments; limited to chemotherapy.

Significance to Biomedical Research and Program of the Institute:

We have identified a group of very potent compounds, the neuropeptides, which exert hormone effects on human monocytes and other cells. Our results, primarily utilizing a chemotactic assay system are novel and indicative of a broader role for these class of compounds in immune system function.

Neuropeptides are known to cause both mood and behavioral alterations when acting within the brain, and to be released into the body during various emotional and physical states. Because these same peptides have very potent effects on macrophages, as well as other components of the immune system, we feel that these compounds are a major class of biochemicals which subserve information exchange between the brain and the body. The functional interaction of the body's cells through networks of neuropeptides and their receptors would be expected to be critical to the health of the organism as a whole and suggests a mechanism by which emotional states can significantly alter the course and outcome of biological illnesses previously considered to be strictly in the somatic realm.

The physiological correlate of in vitro monocyte chemotaxis to these chemicals is still obscure but it seems likely that the local release of neuropeptides may have important effects on cell distribution and activities. Thus, to cite one example, the peptide substance P has been implicated in the vascular erythematouns reactions associated with inflammation and this peptide has very recently been shown to exacerbate experimental arthritis. Monocytes and lymphocytes (which also have substance P receptors) are prominent, locally present, cells which are primarily responsible for the degenerative changes which characterize arthritic lesions. Thus, it seems likely that this neuropeptide, by virtue of its ability to localife and activate immune cells may have an important causative role in this and other inflammatory processes. The recent demonstration that depletion of substance P from the local area surrounding an arthritic joint resulted in a substantial emelioration of the disease suggests the feasibility and importance of a program directed toward the understanding of neuropeptide effects on macrophage and immune function.

The ability of neuropeptides to effect monocyte and some tumor cell migration suggests a further role for these agents in histogenesis and tissue organization, serving to recruit and/or maintain resident macrophage and other cell populations. Disseminated neoplastic diseases may, to some extent, develop as a result of neuropeptide regulated cell trafficking. Thus, tumor cells, which have detached from the primary mass, may respond to organ (site) specific neuropeptide attractants. An understanding of this process could be relevant to controlling tumor spread and may help explain the frequent metastasis of some tumors (e.g., SCLC, breast) to neuropeptide-rich body sites.

Proposed Course:

Explore clinical settings in which neuropeptide macrophage mediated responses may have significant causative or diagnostic potential. Various systemic diseases with an underlying neuropeptidergic component may be reflected in altered macrophage neuropeptide chemotactic responses. An initial survey is being made of illnesses in which macrophages have a role, such as lung cancer to detect such alterations. Other diseases or conditions in which neuropeptides are known to play a role may also reveal alterations at the level of altered macrophage neuropeptide responsiveness. Neuropeptide therapy may prove useful in select illnesses by virtue of effects on macrophage or immune function. *In vitro* systems, such as chemotaxis, could be used to facilitate design of new drugs.

Establish the *in vivo* role for neuropeptides in macrophage function. Neuropeptides can be stimulated to be released at various sites (e.g., electrically, mechanically, chemically) and the accumulation of immune cells studied. These studies would support *in vitro* observations and could suggest the context in which physiological responses may occur. Such information could be important in understanding certain pathological states where macrophages accumulate and in devising ways to regulate their function.

Extended studies revealing antigenic similarities between macrophages and SCLC cells to explore functional similarities. This work will be directed at developing strategems for indutumoring cell differentiation and growth cessation with the aim of developing new therapeutic stategems. These studies will focus on possible lymphokine and monokine regulation of tumor growth/differentiation. We will also attempt to define the conditions which may result in transformation of inflammatory macrophages into cancer.

Biochemical studies aimed at characterizing neuropeptide receptors on monocytes and other immune cells. We will focus on receptor identification through binding and cross-linking studies with the aim of purificaiton and raising antibodies. These studies will make it possible to examine the mechanisms of receptor function leading to cellular activation. Anti-receptor antibodies with agonist on antagonist activity could be used experimentally and possibly therapeutically.

Publications:

1. Rothman, R.B., Herkenham, M., Pert, C.B., Liang, T. and Cascieri, M.A. Visualization of rat brain receptors for the neuropeptide, substance P. Brain Res. 309: 47-54, 1984.

2. Rothman, R.B., Danks, J.A., Herkenham, M., Cascieri, M.A., Liang, T. and Pert, C.B. Autoradiographic localization of a novel peptide binding site in rat brain using the substance P analog, eledoisin. Neuropeptides 4: 343-349, 1984.

3. Ruff, M.R., Wahl, S.M., Mergenhagen, S.E. and Pert, C.B. Opiate receptor mediated chemotaxis of human monocytes. Neuropeptides 5: 363-366, 1985.

4. Ruff, M.R., Pert, C.B., Weber, R.J., Wahl, L.M., Wahl, S.M. and Paul, S.M. Benzodiazepine receptor-mediated chemotaxis of human monocytes. Science, in press.

5. Pert, C.B., Ruff, M.R., Weber, R.J. and Herkenham, M. Neuropeptides and their receptors: A psychosomatic network. J. Immunol. 135: 820S-826S, 1985.

6. Ruff, M.R., Wahl, S.M. and Pert, C.B. Substance P receptor-mediated chemotaxis of human monocytes. Peptides, in press.

7. Hill, J.M., Ruff, M.R., Weber, R.J. and Pert, C.B. Transferrin receptors in rat brain: neuropeptide-like pattern and relationship to iron distribution. Proc. Natl. Acad. Sci. USA 82: 4553-4557, 1985.

8. Gnessi, L., Ruff, M.R., Fraioli, F. and Pert, C.B. Demonstration of receptor-mediated chemotaxis by human spermatozoa: A Novel quantitative bioassay. Exp. Cell Res., in press.

9. Ruff, M.R., Schiffmann, E., Terranova, V. and Pert, C.B. Neuropeptides are chemoattractants for human macrophages and tumor cells: A mechanism for metastasis. Clin. Immunol. Immunopath., in press.

10. Ruff, M.R. and Pert, C.B. Macrophage origin of small cell lung cancer. Technical Comment. Science, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02190-02 NS

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Distribution and Properties of Opiate and Other Brain Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. B. Pert	Pharmacologist	NS, NIMH
Others:	R. J. Weber	Senior Staff Fellow	NS, NIMH
	B. Zipser	Guest Researcher	NS, NIMH
	C. Fraser	Pharmacologist	LNP, NINCDS
	C. Venter	Chief, Recept. Biochem.	LNP, NINCDS

COOPERATING UNITS *(if any)*

Section on Receptor Biochemistry, Laboratory of Neurophysiology, NINCDS.

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Section on Brain Biochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cross-linking is a relatively recent biochemical strategy for covalently affixing reversible ligands to their recognition molecules for subsequent electrophoretic analysis. [^{125}I]-Tyr^{27}-β-endorphin (prepared as originally described by Smythe and co-workers) binds stereospecifically to rat brain membranes. While some studies have suggested that the β-endorphin receptor is a unique "epsilon" opiate receptor, a larger body of evidence suggest that β-endorphin has high affinity for most if not all of the opiate receptors types and subtypes. Cross-linking opiate receptors from different tissue sources can potentially reveal much information about the molecular basis of apparent opiate receptor heterogeneity. Cross-linking, however, only fixes 1% of the bound trace and SDS-PAGE while exquisitely sensitive, can fail to reveal substantial inter-molecular differences. Cross-linking was performed with the homo bi-functional reagent Disuccinimidyl Suberate (DSS). The iodinated cross-linking products of Tetrahymena, leech CNS, and rat brain membranes (both type 1 and type 2 conditions) appeared indistinguishable on SDS-PAGE gel with major cross-linking products at 58K and 100-110K. The strong cross-linked bands produced by incubation in the presence of the inactive opiate ((+)-naloxone) was completely abolished by the same (10^{-6}M) concentration of its active isomer (-)-naloxone. Although we have thus far failed to distinguish between opiate receptors from a mammal, an invertebrate, and a unicellular organism, we continue to explore various conditions of binding, and electrophoresis, (e.g., reduced and unreduced) to examine possible receptor differences, both intra and inter species. Electrophoresis of proteolytic digests of cross-linked bands will be performed as a particularly sensitive method for distinguishing heterogeneity. Thus far, our cross-linking experiment suggest that the recognition molecule (the opiate receptor) which binds all opiate alkaloids and peptides is stable across evolution. As proposed, apparent physiological receptor heterogeneity is due to coupling to other membrane components.

Project Findings:

Objectives:

To map the neuroanatomical distribution of various chemically coded pathway in brain and to understand the neuroscientific significance of "multiple" receptors.

Methods Employed:

Newly developed in vitro autoradiography - unfixed frozen brain tissue is melted onto slides, incubated in appropriate radioactive ligand to label receptors, washed sedially, dried rapidly, fixed with paraformaldehyde vapors and dipped in radiosensitive liquid emulsion for autoradiographic visualization.

Sophisticated computer analysis of receptor binding kinetics is used to rigorously define conditons of multiple opiate receptor binding.

For the first time we bring together rigorous kinetic analysis with autoradiographic distribution of binding sites.

We are cross-linking reversible ligands covalently to their recognition molecule for subsequent electrophoretic analysis.

Major Findings:

One opiate delta receptor appears conformationally fixed, while the other appears capable of assuming mu, delta and kappa conformations.

We showed that β-endorphin labeled opiate receptor from rat, leech and Tetrahymena have the same molecular weights of 58 and 110Kd on SDS-PAGE. This suggests that the opiate receptor is stable across evolution.

Significance to Biomedical Research and Program of the Institute:

Pinpointing neurochemically coded tracts will enable us to determine the functional significance of each newly discovered pathway. The method can be used on human brain and ultimately should give information about the contribution of various neurochemically coded tracts to pathology.

Proposed Course:

We plan a sophisticated biochemical and immunological approach to further defining the molecular nature of opiate receptors. The type 1 opiate receptor complex with its advanced evolutionary accumulation in the forebrain of humans seems particularly worthy of further study (see Project Number Z01 MH 02182-03 NS, Toward the Visualization of Opiate Receptors in Living Human). We plan to study the brain distribution of insulin, transferrin, and their receptors to further demonstrate the breakdown in the distinction between "neuropeptides" and hormones.

Publications:

1. Clarke, P.B.S., Pert, C.B. and Pert, A. Autoradiographic distribution of nicotine receptors in rat brain. Brain Res. 323: 390-395, 1984.

2. Rothman, R.B., Schumacher, U.K. and Pert, C.B. Effect of beta-FNA on opiate delta receptor binding. J. Neurochem., 1197-1200, 1984.

3. Clarke, P.B.S., Schwartz, R., Paul, S.M., Pert, C.B. and Pert, A. Nicotinic binding in rat brain: autoradiographic comparison of [^{3}H]acetylcholine, [^{3}H]nicotine, and [^{125}I]-α-bungarotoxin. J. Neurosci. 5: 1307-1315, 1985.

4. McLean, S., Skirboll, L.R. and Pert, C.B. Comparison of substance P and enkephalin in rat brain: an overview using radioimmunocytochemistry. J. Neurosci. 14: 837-852, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02146-06 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Etiology of Problem Aggression in Early Childhood

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Mark Cummings	Staff Fellow	LDP/NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP/NIMH
	Ronald J. Iannotti	Research Psychologist	LDP/NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~XXXXXXXX~~ Person Years	PROFESSIONAL:	OTHER:
.95	.90	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One problem with remediation programs for problem aggression may be that they are begun too late, after aggressive styles have become highly stable elements of personality, resistant to change. This research attempts to lay the ground work for earlier prevention and intervention by studying the origins of highly aggressive behavior, and the factors that contribute to the development of aggression and other emotional or behavior problems. Children are studied at one year of age, and followed up at school-age. Two main findings have emerged: (1) There is evidence for an aggressive syndrome in young boys, marked by sensitivity to environmental stress, high intensity of aggression, labile emotions, and high stability of aggression over time and in multiple settings. (2) Anger or conflict between adults acts as a powerful instigator of aggression and other emotional and behavioral problems. Responding to background anger is stable from an early age, but is modifiable by experience, with repeated exposure resulting in increased sensitization and greater aggressiveness. Emotional reactivity to background anger increases with age, but so does the range and flexibility of coping patterns.

Project Description:

Aggressive, angry, or hostile individuals pose a serious threat to the safety and welfare of others, but efforts at remediation have not proven to be highly successful, perhaps because aggressive individuals are identified and placed into treatment too late. This research attempts to lay the groundwork for earlier prevention and remediation by identifying: (a) early aggressive patterns and the extent to which they are predictive of later aggressiveness, and (b) elements of the early environments that promote the formation of aggressive patterns and other emotional and behavioral problems.

The procedures are described in earlier reports. In brief, children are studied at two years of age, with some children followed up at school-age. Styles or patterns of aggression are studied in the home, and specific hypotheses regarding determinants of aggression are tested in the laboratory.

Two main findings have emerged. (1) There is evidence for an aggressive syndrome in young boys, characterized by high intensity aggression, labile emotions, and sensitivity to environmental stress. Findings suggest that a dimension of temperament may underlie this syndrome. These trends are currently being further analyzed at school-age.

(2) Anger or conflict between adults (background anger) powerfully affects young children. Exposure to background anger has been shown to influence overt and covert emotional responding, have immediate and delayed effects on behavior, influence behavior towards actors and bystanders, and influence aggression and the intensity of aggression. The quality of reactions to background anger is relatively stable from an early age, but is modifiable by experience, with repeated exposure resulting in increased sensitization. Emotional reactivity to background anger increases with age, but so does the range and flexibility of coping patterns. These findings have been replicated in several studies. After exposure to background anger children show more frequent aggressive behavior, and are more prone to intense outbursts of aggression.

The findings of these studies suggest that well-delineated and stable aggressive syndromes may be present in very early childhood, and that, consequently, early childhood may be an optimal time for intervention. The results also suggest a significant and general role of anger in the environment as a risk factor for deviant development.

Significance for Biomedical Research

This research provides partial answers to the following clinically significant issues: (1) What responses identify children to be at risk for problem aggression and what is the prognosis? (2) What is the role of background anger as an instigator of aggression and other behavioral and emotional disturbances?

Proposed Course

Final coding, analyses, and manuscript preparations are underway. This is a final report.

Publications:

Cummings, E.M., Iannotti, R.J., and Zahn-Waxler, C. The influence of conflict between adults on the emotions and aggression of young children. Dev. Psychol., 21: 495-507, 1985.

Cummings, E.M., and Beagles-Roos, J. Toward a model of infant day care: Studies of factors influencing responding to separation in day care. In Ainslie, R. (Ed.), The Child and the Day Care Setting. New York: Praeger, 1985, 159-182.

Cummings, E.M., Zahn-Waxler, C., Iannotti, R.J., Hollenbeck, B., and Radke-Yarrow, M. The Early Organization of Individual Differences in Aggression and Altruism. In Zahn-Waxler, C., Cummings, E.M., Iannotti, R.J. (Eds.), Development of Altruism and Aggression, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02150-06 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adjustment to Stress in Early Adolescence: Environmental & Organismic Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Editha D. Nottelmann	Guest Researcher	LDP/NIMH
Other:	C. Jean Welsh	Research Psychologist	LDP/NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MANYEARS: Person Years	PROFESSIONAL:	OTHER:
.30	.00	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of major transitions on psychological adjustment is investigated in early adolescence. Children are studied across the year of transition from elementary school to middle school and junior high school, which for many children coincides with the onset of puberty. The focus is on the effect of imposition of multiple change. Analyses examine change and stability in self-concept and self-esteem, and their correlates, in relation to physical growth indices of physical maturity (rate of growth, body build, height/weight ratio) across the year of transiton.

Project Description:

The study examines the effect of major transitions on early adolescent adjustment. A large group of children is studied across the year of transition from elementary to middle and junior high school, which represents significant imposed change: change in schools, change in academic demands, and change from child to adolescent status. For many children, the timing of these transitions coincides with the onset of puberty. Most children are able to negotiate one developmental task at a time. When school transition, social transition, and the onset of puberty converge to impose several changes at the same time, however, they are at risk for adjustment problems.

Among the subgroups of children identified as potentially at risk are children with aggressive tendencies (61 boys and 21 girls), representing 18% of our sample. As previously reported, they had significantly lower self-esteem and were less competent academically (according to self-ratings and teacher ratings) than children in matched control groups. In addition, "aggressive" children had less positive perceptions of their school environment; that is, they reported less student involvement, less teacher support, less rule clarity, and less organization in their school than the control group. They also were less affiliative; that is, they nominated fewer classmates, when asked to identity those with whom they would like to engage in "play" and "work" actvities, than did children in the control group. There were no clear-cut differences, however, in the number of friendship nominations that "aggressive" and control group children received from their peers.

Another subgroup consists of 126 children (66 boys, 60 girls) identified as either short or tall for their grade (one or more standard deviations below or above mean height, respectively, for boys and girls in their grade), representing 29% of our sample. The findings, which were stronger for girls than for boys, suggest that self-perceptions (self-esteem and perceived social competence) change with the social environment provided by schools. The lowest self-ratings were those of tall girls in elementary school and short girls in secondary school.

Significance for Biomedical Research:

The research provides normative data for psychological adjustment in early adolescence, which is an under-researched period of development. It is documenting both incidence and types of marginal adjustment that are likely to be precursors of serious adjustment problems in later adolescence. The findings are of value to child psychiatrists and pediatricians.

Proposed Course:

Manuscripts are in preparation. This is a final report.

Publications:

Nottelmann, E.D., & Welsh, C.J. The long and the short of physical stature in early adolescence. Journal of Early Adolescence, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02152-06 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Discipline and Parental Control in Families with Affective Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Leon Kuczynski	Visiting Associate	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	Grazyna Kochanska	Guest Researcher	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Affective Development

INSTITUTE AND LOCATION

NIMH, Building 15K, 9000 Rockville Pike, Bethesda, Maryland 20205

TOTAL ~~MANYEARS~~ Person Years	PROFESSIONAL:	OTHER:
.70	.35	.35

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Children's compliance and Parental discipline and control practices in families with normal and clinically depressed mothers are investigated. This study, the basic paradigm for which is described in Annual Report MH 02144, is part of a series of investigations assessing the environmental transmission of competent and disordered patterns of child behavior in families with normal and affectively disturbed parents. Impaired parental skills in managing children's behavior have repeatedly been implicated in the etiology of maladaptive patterns of child behavior, such as noncompliance, aggressiveness, and other antisocial behaviors.

Assessments of parent and child behavior are based on detailed observations of parent-child interaction in a naturalistic setting. Measures of parental control include the goals of parental intervention, the quality and timing of mothers' strategies and their ability to resolve conflicts successfully. Measures are also taken of children's compliance and resistance to parental attempts to influence their behavior.

Preliminary comparisons between the two diagnostic groups indicated that toddlers of depressed mothers are at greater risk for compliance problems than children of normal mothers.

For toddlers of normal mothers there was an increasing trend with age for children to comply with parental requests but not for children of depressed mothers. The data imply that depressed mothers may be relatively ineffective in controlling children's behavior and that this difficulty may increase with age of child.

Project Description:

The determinants, contents and effects of parental discipline and control practices in families with normal and clinically depressed mothers are investigated. This study, the basic paradigm for which is described in Annual Report #MH 02144, is part of a series of investigations assessing the environmental transmission of competent and disordered patterns of child behavior in families with affectively disturbed parents. Several studies suggest that depressed mothers are less involved in the day-to-day control of their children and, when they do intervene, use punitive forms of discipline. Impaired parental skills in managing children's behavior have repeatedly been implicated in the etiology of maladaptive patterns of child behavior such as noncompliance, aggressiveness, and other antisocial behaviors. There is also evidence that child noncompliance in turn may exacerbate parental feelings of depression. However, existing assessments of parent and child behaviors have either been global in nature or have focused on economically stressed populations and limit the conclusions that can be drawn.

One purpose of this study is to provide a detailed behavioral assessment of the control practices of depressed and nondepressed mothers and to examine the effects of their practices on children's self-control, compliance, and regulation of emotions. Specific aspects of parental interventions that are being assessed include the timing of the intervention (e.g., whether the mother intervened early to prevent an anticipated, potential misbehavior or late, in reaction to a misbehavior that has already occurred), the issue involved (which behavior the parent attempts to control), parental adaptation of strategies to different kinds of children's misbehaviors, and the outcome of the parent's intervention (whether the parent ultimately succeeds or fails to elicit the child's cooperation). Parental influence strategies are analyzed in terms of their separate verbal, physical and emotional components. The influences of social class of family and of the current status and seriousness of mothers' affective disorder and developmental level of the child are examined. An important question is whether difficulty in the management of children's behavior is an enduring feature of depressed mothers or whether it consists of transient problems that are confined to the acute stages of the depressive episode.

A preliminary analysis of the currently available data was performed on a sample of 25 children of normal mothers and 31 children of depressed mothers in order to investigate developmental changes in children's reactions to their mothers' attempts to influence their behavior. Half the children in each group were younger toddlers of mean age 26 months and half were older toddlers of 39 months.

For the sample as a whole, the findings indicate that there were qualitative developmental changes in the form of children's resistance to parental limit-setting and control. Overt noncompliance accompanied by defiance and anger decreased with age during toddlerhood, whereas indirect forms of noncompliance such as attempts to delay compliance, bargain or modify parental demands

increased with age. It was also apparent that parental use of power assertive strategies such as direct commands and physical enforcement were related to overt defiance, whereas indirect parental strategies, suggestion and reasoning were related to indirect forms of active resistance.

Comparisons between the two diagnostic groups indicated that children of depressed mothers were at greater risk for problems with noncompliance than children of normal mothers. For toddlers of normal mothers there was an increasing trend with age for children to comply with parental requests but not for children of depressed mothers. Children of depressed mothers were more likely to respond with passive noncompliance to parental attempts to influence their behavior, and more so than children of normal mothers, showed an increasing trend with age to actively delay compliance.

These data imply that depressed mothers are relatively ineffective in controlling their children's behavior and that this difficulty increases with age of child. A hypothesis that will be explored in future analyses is that depressed mothers may be less assertive than normal mothers in their exercise of control and less likely to enforce parental limit-setting in the face of resistance from their children.

Significance to Biomedical Research:

Children of depressed parents are at greater risk for psychopathology and behavioral disorders than are children of normal parents. Research on child development has demonstrated that aberrant parental disciplinary practices are important contributors to children's disordered social and emotional development. How depression affects the parent's abilities to function in controlling child behavior is largely unresearched; yet this variable may contribute significantly in creating a pathogenic environment for young children.

Proposed Course:

Data have been collected on 100 families. An instrument for detailed coding of naturalistic childrearing control episodes has been developed and tested. Coding of one hour of videotaped data for 75 families has been performed; an additional six months of coding time is anticipated.

Publications:

Kuczynski, L.: Socialization goals and mother-child interaction: Strategies for long-term and short-term compliance. Dev. Psychol. 20: 1061-1073, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02155-06 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Children of Depressed and Normal Parents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	E. Mark Cummings	Staff Fellow	LDP NIMH
	Ronald Iannotti	Research Psychologist	LDP NIMH
	Penelope Trickett	Guest Researcher	LDP NIMH
	Karen Barrett	Guest Researcher	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Child Behavioral Disorders

INSTITUTE AND LOCATION

NIMH, Building 15K, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205

TOTAL ~~MAN-YEARS~~: Person Years	PROFESSIONAL:	OTHER:
1.60	.90	.70

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research is to identify dimensions of child and family functioning that influence aggression, depression, and social withdrawal in children. Psychiatric interviews and observational measures of 5- to 6-year-old children's social skills and deficits in peer interactions are used to identify these problem areas. Naturalistic and experimental assessments of these children and their families three years earlier permit detailed exploration of parent and child factors early in development that may predict the later occurrence of diagnosable disturbance. Examples of child risk factors include early deficits in social skills, difficulties modulating affect in stressful situations, submissiveness, and intense aggression. Examples of parent risk factors include depression and family discord, deviant parental social interaction patterns and affective communication styles. The follow-up analyses are underway.

Project Description:

The purpose of this research is to identify dimensions of family and toddler-child functioning that are predictive of emotional problems when the child reaches school age. Internalizing disorders or problems of overcontrol (depression, guilt, social withdrawal) and externalizing disorders or problems of undercontrol (aggression, conduct problems) are investigated in a sample of six-year-olds. Structured psychiatric and psychological tests are used. Similar patterns of behavior are evaluated also in observational assessments of the children's relationships with their peers. The emotional problems of six-year-olds are examined in relation to children's social orientations and affective coping styles that were measured at two years of age. Parental variables that might be expected to influence aggression and depression in children, and hence to influence continuities/discontinuities over time in children's emotional problems are also assessed. The parental and familial influences explored include: (1) the nature of the parents' direct interactions with the child, (2) the parents' mental health status, and (3) the family climate and relationships.

In our earlier studies of two-year-olds, parental depression was related both to problems of overcontrol and undercontrol in children. The longitudinal assessments now make it possible (a) to determine whether and how these problems in children carry over to the school years and (b) to explore additional factors of development and environment that contribute to these disturbances. Two related studies are also being conducted. One, a methodological investigation, examines the accuracy of maternal retrospective self-report data regarding parenting practices and problems of children. Retrospective parental reports are often used to obtain diagnostic and child-rearing information on children (and this is one data source used in the present longitudinal study as well) [see 02153-05 for details of the retrospective project].

A second study uses a new sample of two-year-old children to measure early signs of guilt and shame. There are indications that these affects emerge in the second and third year of life, but research is needed to determine (a) how they can be objectively measured and (b) the conditions under which they are likely to be adaptive emotions rather than precursors of later emotional problems.

Methods employed and major findings:

Forty-eight two-year-old children were seen in three laboratory sessions, each lasting 1 - 1 1/2 hours in length and spaced two weeks apart. Children of both normal and depressed mothers (SADS-L) were studied. Children were exposed to a range of challenging and stimulating conditions in order to evaluate their social and emotional interchanges with the mother, with familiar playmates and with an adult stranger. Assessments were made of the child's ability to sustain social play, compete adaptively for resources, "negotiate" problems, cooperate, cope with frustration without resorting to intense aggression, empathize, and so on. Children's social problem-solving abilities were assessed also in hypothetical situations. Maternal characteristics relevant to children's social skills were also evaluated; namely, maternal sensitivity (Ainsworth), supportive presence

and quality of assistance (Sroufe), and specific techniques used to encourage cooperation and sustained social and task-oriented involvement with others. At age five children were seen again. Areas of functioning parallel to those evaluated in the two-year testing were assessed. Diagnostic information was obtained on mother, father and child. Child-rearing practices and the marital relationship were also measured. At age six the Childhood Assessment Schedule was used to obtain psychiatric diagnoses on the children and to assess areas of concern. The mothers completed the Achenbach Child Behavior Check List and provided data on family functioning, using the Family Adaptability and Cohesion Scale.

Significance to Biomedical Research and the Program of the Institute:

A basic aim of prevention and intervention research is to identify early in development those child and family factors that contribute to later childhood disturbances. Childhood depression and anti-social behavior both usually begin to be diagnosed when children reach school age, but the causes may have much earlier origins. If early contributing factors can be isolated, the design and implementation of more scientifically based intervention procedures would be facilitated.

Proposed Course:

Data collection has been completed; most of coding has been completed. Data analysis will be well underway in the coming year.

Publications:

Zahn-Waxler, C., Cummings, E. M., Iannotti, R. J., and Radke-Yarrow, M. Young offspring of depressed parents: A population at risk for affective problems. In D. Cicchetti and K. Schneider-Rosen (Eds.), Childhood Depression, New Directions for Child Development, no. 26. San Francisco: Jossey Bass, December, 1984, 81-105.

Zahn-Waxler, C., Chapman, M. and Cummings, E. M. Cognitive and social development in infants and toddlers with a bipolar parent. Child Psychiatry Hum. Dev. Vol. 15(2), Winter, 1984, 75-85.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02156-06 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Personality Development of Children Reared by Normal and Depressed Mothers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marian Radke-Yarrow	Chief	LDP NIMH
OTHER:	Grazyna Kochanska	Guest Researcher	LDP NIMH
	Tracy Sherman	Senior Staff Fellow	LDP NIMH
	Leon Cytryn	Medical Officer, Psychiatry	LDP NIMH
	Donald H. McKnew, Jr.	Medical Officer, Psychiatry	LDP NIMH
	Leon J. Kuczynski	Visiting Associate	LDP NIMH
	Barbara Hollenbeck	Social Science Analyst	LDP NIMH

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~XXXXXXXX~~: Person Years	PROFESSIONAL:	OTHER:
1.75	.40	1.35

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two areas of social and affective development are being investigated in offspring of normal and depressed (major and bipolar) mothers. Children are studied at 2 to 3 years of age and siblings at 5 to 8 years of age. They are followed-up three years later. Inhibited behavior and signs of affective (dysthymic or depressed) disturbance are studied. Inhibited behavior in the face of the unfamiliar (places, persons) is observed in semi-naturalistic but standard settings, at both periods of measurement. Based on very preliminary findings, children's inhibited behavior does not appear to differ by diagnostic category of parent. However, normal mothers tend to use positive expressions to help the child in the face of distress and inhibition whereas depressed mothers respond negatively. To assess the affective status of the children, psychiatric assessments of 2- and 3-year-olds were made using a play interview. A structured psychiatric interview was used with the 5- to 8-year-olds. There is little difference by diagnostic group of the mother in the assessments of the younger children, but among the school-age children, dysthymic and depressed affects are more frequent in the offspring of depressed women than in the offspring of the well mothers.

PHS 6040 (Rev. 1/84) GPO 914-916

Project Description:

The personality development of children of normal parents and major and bipolar depressed parents is investigated. Research has demonstrated increased likelihood of affective problems in the offspring of depressed parents. In most of the studies, however, the offspring have been adolescents or adults. There is little information concerning young children of depressed parents, or concerning behavioral characteristics prior to the onset of clear mood disorders. In the present research, two siblings, age 2 to 3 and 5 to 8 years, from 120 families, are studied. Two areas of social and affective development are being examined:

Study A. Tendencies toward withdrawn, inhibited behavior and/or acting-out, aggressive behavior represent troubling signs in children of any age. Their appearance in 2- and 3-year-olds is especially indicative of risk since there is some evidence of relative stability of these patterns over time, and since they are linked with other forms of emotional and behavioral problems. Both of these attributes might be expected to have an increased likelihood of occurrence in offspring of depressed parents.

Methods

Inhibition is studied by observing child behaviors in the face of the unfamiliar. Unfamiliarity is created by semi-naturalistic but standard settings in which the child enters an unfamiliar environment, is introduced to and interacts with a strange adult, and with a strange peer. The child's behaviors are studied in terms of approach/withdrawal towards the novel, strategies used to handle and explore the unfamiliar, affective reaction and emotional dependence on the mother. Mother's strategies are studied including behavioral and affective reactions that may facilitate or hinder child's coping.

Inhibition in the face of the unfamiliar is studied at two points in time: at 2 to 3 years and 5 to 6 years of age with mothers present and free to interact with their children. Consistency across settings and stability across time are being assessed.

Findings

Preliminary findings (on a third of the sample) show no differences in toddler-age children of normal and depressed mothers. However, the affective interplay between mother and child appears different for normal and depressed groups. When the children display inhibition in encountering the unfamiliar, normal mothers use positive affective expression to help them. The pattern seems different in dyads with a depressed mother. For this group, a toddler's expression of distress and inhibition in the face of the unfamiliar brings negative affective expression from the mother.

(Investigation of acting-out behaviors is in a planning stage, and not reported this year.)

Study B. Extrapolating from the evidence on offspring of older ages, young children of depressed parents are likely to manifest more affective and

behavioral disturbance than are the offspring of normal parents.

Methods:

Each child was seen individually by a psychiatrist. The toddler was seen in a play interview; the child was rated on areas of concern (dysthymic, depressed, overanxious, control of temper, relationship problems, separation problems, excessive mood changes, developmental delay, personality problems). The 5- to 8-year-old sibling was given a structured psychiatric interview (the Child Assessment Scale) and rated for the same areas of concern. The psychiatrists were blind to the parents' diagnoses. Each child was seen by a different clinician. Assessments are being repeated in the follow-up study, three years after the first assessment. The structured interview is used. The child was not seen by the same clinician as in the initial assessment.

Findings:

Based on approximately 75% of the sample, findings are as follows: When any area of concern is counted for the 3-year-old, the offspring of normal, major depressive, and bipolar parents show no differences in frequency of disturbance. Somewhat more than a third of the cases are rated in one or more areas of concern. When only dysthymia and depression are considered, only one child (out of 50) in the group of major depression is so rated. When any area of concern is rated for the 5- to 8-year-old siblings, the frequency in normal, major depressive, and bipolar groups is 45%, 53%, and 71%, respectively. When the same groups are rated only for dysthymia or depression, the frequencies are 21%, 40%, and 50%, respectively.

Significance to Biomedical Research:

A developmental picture of affective disorders does not exist. Identification of early signs of disturbance and factors associated with such disturbance will provide better understanding of affective illness, and will offer information on how to plan intervention.

Proposed Course:

Each of the studies is midway. Analyses will continue in the inhibition study. The coding of acting-out behavior will be developed. The psychiatric assessments will be examined in greater depth, as one of several diagnostic child assessments. Parental pathology will be considered not only by diagnosis but with regard to genetic history, symptomatic behavior, and current treatment of parent.

Publications:

Radke-Yarrow, M. and Zahn-Waxler, C.: Familial factors in the development of socially valued behavior. In Block, J., Olweus, D. D., and Radke-Yarrow, M. (Eds.): Aggression and Socially Valued Behavior: Biological and Cultural Perspectives. San Diego, CA: Academic Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02158-06 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Impact of the Environment on the Development of the Abused Child

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Penelope K. Trickett	Guest Researcher	LDP NIMH
Other:	Elizabeth J. Susman	Senior Staff Fellow	LDP NIMH
	Leon Kuczynski	Visiting Associate	LDP NIMH
	Malcolm Gordon	Research Psychologist	LDP NIMH

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20205

TOTAL MANYEARS: Man Years	PROFESSIONAL:	OTHER:
1.30	1.20	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study focuses on the emotional development of physically abused children and the relation between this development and the childrearing environment of the home. The child's development is assessed in relation to cognitive and physical maturity, affective behavior, interpersonal problem solving skills, and behavior problems. The childrearing environment is assessed in terms of psychosocial environment of the home, family social network and social supports, parental frustration tolerance, childrearing attitudes and practices, and parental mood. Psychopathology in the parents is also assessed by the Schedule for Affective Disorders and Schizophrenia. Subjects are 4- to 10-year-old abused and control children and their families.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

This study focuses on the psychological and behavioral development of physically abused children and the relation between this development and aspects of the rearing environment of the child including the presence of psychopathology in the parents. Childhood victims of physical abuse are known to be at risk for later behavioral maladjustment. There are few systematic data on their development. Likewise, little research exists concerning the processes within the family that lead to abusive incidents. What contribution parental psychopathology may play in triggering abusive episodes is also unknown. The relations between the childrearing variables and the development of the abused child are examined. The child's development is likely to be affected not just by the sporadic episodes of physical abuse, per se, but by the more enduring childrearing environment of the home.

Participants are physically abused children ranging in age from 4 to 10 years and their parents. They are recruited from protective service agencies in the Washington Metropolitan area. A control group of nonabusing families was recruited from community agencies. The total sample is 56 families.

Standardized measures are used to assess the child's cognitive and physical functioning, social problem solving skills, and behavior problems. Affective coping style and predominant mode of relating to family members are measured by observational methods. To assess the childrearing environment, a combination of standardized measures and observational methods is used. The variables include family psychosocial environment, parental frustration tolerance, parental childrearing attitudes, values and practices, parent-child interaction, parental mood, and parental psychiatric diagnosis (SADS). (See previous years' reports for details.)

Major Findings:

The findings show subtle and pervasive differences in attitudes about childrearing between abusive and nonabusive parents. Abusive parents also do not report using physical punishment more often than do control parents but are more likely to report using more severe forms of punishment: striking the face, hitting with an object or pulling hair. Thus, there is a qualitative rather than a quantitative difference in the types of punishment used by abusive and nonabusing parents. Besides these differences in discipline attitudes, there are differences in the two groups in emotional suppression and expression. While the abusive parents are more likely to express anger, aggression and conflict than control parents, they appear more likely to suppress other types of emotional expression, both positive and negative. Other differences between these groups seem related to the isolation of abusive families from the world outside their families. The abusive families were lower than the control families on their degree of engagement with the larger community. They failed to engage in recreational activities or to attend cultural events. The psychological component of this isolation is reflected in their discouragement of new and independent experiences for their children. Abusive families appear to find the outside world to be a hostile place and believe they must protect themselves from the threat of their community. In summary, abusive parents are not extremely

different on any one psychological characteristic. Rather the aberrant quality of the abuse group becomes apparent only when the set of findings is considered as a totality.

Significance to Biomedical Research:

This study addresses two distinct etiological issues. One focus is on the causes of child abuse with particular emphasis on the role played by parental psychopathology and parental child-rearing attitudes and behavior. The second focus is on the effect of child abuse on the psychological development of the child victims. Information on these two issues can assist in the development of treatment programs for abusive families and preventive interventions.

Proposed Course:

Data collection is complete. The coding and analysis of the data continue. Manuscripts are in press and others are in preparation. This is a final report.

Publications:

Susman, E. J., Trickett, P. K., Iannotti, R. J., Hollenbeck, B. F., and Zahn-Waxler, C. Child-rearing Patterns in Depressed, Abusive, and Normal Mothers. Am. J. Orthopsychiatry, 55: 237-251, 1985.

Trickett, P. K. and Kuczynski, L. Children's Misbehaviors and Parental Discipline Strategies in Abusive and Non-abusive Families. Dev. Psychol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02159-05 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Information Processing and Adaptation to Research Hospitalization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Elizabeth J. Susman	Senior Staff Fellow	LDP/NIMH
Other:	Lorah D. Dorn	Social Science Analyst	LDP/NIMH
	John C. Fletcher	Special Assistant	CC/DIR

COOPERATING UNITS *(if any)*

Office of the Director, Clinical Center

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~MANYEARS~~: Person Years	PROFESSIONAL:	OTHER:
.45	.10	.35

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study focuses on the development of children's reasoning about disease and treatment processes. Because children may fail to understand the abstractions related to disease and treatment, their participation in research has always presented special challenges. The role of anxiety, perception of control over illness, and stage of cognitive development were examined as possible influences on reasoning about illness. Standard measures of cognitive functioning, interview measures designed to assess reasoning about one's own illness and illness in general, and a measure of anxiety were administered to the participants. These measures permit exploration of developmental differences in comprehension of one's medical situation, and allow examination of the effects of anxiety and perception of control over illness on comprehension at different age levels. Participants are child, adolescent, and adult inpatients and a comparison group of healthy participants. Findings show that cognitive as well as emotional factors are related to child and adolescent reasoning about illness and treatment. Older and more cognitively mature children had a higher stage of reasoning about their own illness and illness in general. Anxiety level was not related directly to stage of reasoning about illness. However, children who were higher on anxiety level were lower on perception of control over illness. Promoting a sense of control over illness may help children cope with the stress that accompanies major illnesses.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

This study focuses on the development of children's reasoning about disease and treatment processes. Children's participation in medical research has always presented special challenges. What is their level of understanding of the disease and the treatment process and how is their understanding affected by their anxiety concerning hospitalization? How should their assent be interpreted in light of their various levels of understanding? This study focuses on a part of these interlocking issues (a) by comparing levels of reasoning of children and adolescents, (b) by examining level of reasoning of the disease and treatment processes in relation to standard measures of cognitive functioning - unrelated to disease, and (c) by assessing level of anxiety and perception of control over illness. A more general question, the effects of stress and anxiety on cognitive functioning, is addressed in the comparisons of the patient's level of cognitive functioning on the non-stressful content of the standard cognitive tests with his/her comprehension and reasoning on the stressful medical content. Participants are child, adolescent, and adult inpatients and a comparison group of healthy participants.

Participants in the study are children (7-12 years), adolescents (13-18 years), and young adults (19-30 years) who are admitted to the Clinical Center for the first time. The patients are enrolled in medical protocols involving either the treatment of obesity or the treatment of childhood cancer. A comparison group of healthy non-hospitalized participants matched for age, gender and socioeconomic status also is included. Seventy participants are in the study. Level of reasoning and understanding about their illness and treatment regimens is obtained through interviews. Standard tests of cognitive abilities and reasoning about non-stressful content also are administered. Anxiety is measured using the Spielberger State-Trait Anxiety Scale. Participants are retested after 6 months.

The findings show that chronological age, cognitive developmental stage and perception of control over illness all are important components in the development of children's and adolescent's understanding of illness and treatment. Those children who were older and more cognitively mature were at a higher stage of reasoning about illness than younger and less mature children. In addition, children who were higher on perception of control over their own illness were at a higher stage of reasoning about their own illness and illness in general. The anxiety level of children was not related directly to reasoning about illness. However, children who were high on anxiety levels were lower on perception of control over illness. And the lower children were on perception of control over illness, the lower they were on stage of reasoning about illness. Perception of control may moderate relations between anxiety and reasoning about illness. Promoting a sense of control over illness may help children cope with the stress that accompanies major illnesses.

Significance to Biomedical Research:

Comprehension of a disease and treatment and level of anxiety are significant factors in the behavior problems and noncompliance frequently

observed in hospitalized children and adolescents. Systematic data on these factors can aid in developing effective methods of communicating complex medical information to children.

Proposed Course

Manuscripts are submitted for publication.

This is a final report.

Publications:

Susman, E.J. and Hollenbeck, A.R.: Sequential variations in the interactions between caregivers and child and adolescent cancer patients. International Journal of Behavioral Development, 7, 395-421, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02161-05 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Changes in Imitative Learning

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Leon Kuczynski	Visiting Associate	LDP NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Affective Development

INSTITUTE AND LOCATION

NIMH, Building 15K, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205

TOTAL ~~MANYEARS~~ Person Years	PROFESSIONAL:	OTHER:
.55	.35	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of children's imitative behavior is investigated in the natural environment. Data were obtained on 24 children over a nine-month period during the second and third years of life. Sources of data consisted of descriptive accounts of imitation by mothers trained in observational recording. Although most of the incidents of imitation consisted of immediate repetitions of the behavior of models, incidents of delayed imitation increased from late infancy to early toddlerhood. Several developmental changes in the content of children's imitations also occurred during this period. The imitation of affective behaviors and of noninstrumental behaviors decreased as children grew older. Increases with age were found for caretaking, self-care, and household task behaviors, other-directed discipline and control behaviors, social interaction behaviors, and mannerisms and expressive characteristics of models. The overall pattern of findings suggests that imitation is an important process in the early acquisition of competent behavior patterns and that imitation of conventionally competent social and instrumental behavior patterns increases with age.

Project Description:

The early development of children's imitative behavior in the natural environment is investigated. Children's imitation of parents, siblings, and other models is considered an important process in children's acquisition of complex patterns of behavior. Previous laboratory research with pre-school and older children suggests that aggressive, prosocial, self-controlled, and impulsive patterns of behavior may be promoted by imitation. However, information is lacking about the kinds of behaviors relevant to socialization that children spontaneously imitate in the natural environment and about the developmental and environmental processes that mediate the role of imitation in children's learning in the first years of life.

The present study extended previous research by investigating the development of both immediate and delayed forms of imitation as it occurs in natural settings. The data consisted of narrative descriptions of 1,459 incidents of imitation collected over a 4- to 8-month period by mothers who were trained in observational recording. Twelve children were 16 months old and 12 were 29 months old during the middle of the data collection period. The data were transcribed from audiotapes and coded in terms of the identity of the model (parents, peers), timing (immediate or delayed) and 9 content categories including affective behaviors, expressive behaviors, discipline/control behaviors, conventional social and instrumental behaviors and behaviors that had no conventional social or instrumental meaning. Analyses were performed to investigate the effects of age of child and the identity of the model for the imitation on the content of children's imitations.

Age changes in children's imitations were found and were indicative of cognitive and motivational development taking place during the second year of life. Most of the incidents consisted of immediate imitations. However, the proportion of the total made up by delayed imitations increased from 16 to 29 months and indexed toddlers' growing capacity for symbolic representation.

In terms of content, imitations of emotional behaviors and of noninstrumental behaviors decreased with age. Increases with age were found for imitation of instrumental behaviors such as caretaking, self-care and household tasks; conventional interpersonal behaviors and mannerisms; and expressive characteristics of the model. Theoretically, these changes suggest that the social meaning of a behavior, rather than novelty or complexity, increasingly determines children's imitations after infancy. Practically they suggest that children become selective in the behaviors they imitate with age and increasingly focus on competent features of the model's behavior and on behaviors that are meaningful from the standpoint of socialization.

More detailed analyses of two categories of imitation -- parental discipline and emotions -- also indicate a possible development from simple, discrete repetitions to more organized, functional patterns of behavior. For example, although there were no overall age differences in children's imitation of parental behaviors during disciplinary interventions, there was a change in the function of these imitations. Whereas younger children were more likely simply to repeat parental reprimands without directing them to others, older children were more likely to direct their imitations to others. Similarly,

although imitations of emotions, in general, decreased with age, there was an increase in the extent to which these imitations evidenced a deliberate, contrived quality -- a development that may make possible the instrumental use of affect in interpersonal communication.

Finally, the content of children's imitations was found to depend on whether the original model of the behavior was the parent or a sibling or peer. A greater proportion of imitations of parents consisted of chores/caretaking/self-care behaviors and of discipline/control behaviors, whereas imitations of peers more often consisted of affective behaviors and miscellaneous noninstrumental behaviors. Finally, delayed imitations made up a greater proportion of parents than of peers. To the extent that delayed imitations are an index of the behaviors that are at least temporarily retained by children, this suggests that parents may have a greater impact than peers in children's early imitative acquisition of basic social and instrumental competencies.

In summary, this study suggests that imitation may be a fundamental process in children's personality development and particularly in the acquisition of instrumental and social skills. Moreover, the patterns of developmental changes indicate that children's imitation of conventionally competent patterns of behavior increases from late infancy to early toddlerhood.

Significance to Biomedical Research:

Imitation is a basic process of learning and has obvious implications for the environmental transmission of complex patterns of behavior. An inherent aspect of the environment of children living with parents suffering from psychopathology is the presence of models of disordered patterns of behavior and affective expression. Although few studies have investigated directly what is learned by imitation, disordered forms of parental behavior are thought to be transmitted intergenerationally by this process. This study makes a start in assessing the impact of parent and sibling models and by examining the kinds of behaviors that are susceptible to imitation.

Proposed Course:

A report of these findings has been submitted for publication in Developmental Psychology.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02164-05 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Impact of Biological Changes on Psychological Functioning During Adolescence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Elizabeth J. Susman	Senior Staff Fellow	LDP/NIMH
Other:	Editha D. Nottelmann	Guest Researcher	LDP/NIMH
	Gale E. Inoff	Research Psychologist	LDP/NIMH
	Lorah D. Dorn	Social Science Analyst	LDP/NIMH
	George P. Chrousos	Senior Investigator	DEB/NICHD
	Gordon B. Cutler	Senior Investigator	DEB/NICHD
	D. Lynn Loriaux	Chief	DEB/NICHD

COOPERATING UNITS *(if any)*

Developmental Endocrinology, NICHD

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~MANYEARS~~ Person Years	PROFESSIONAL:	OTHER:
1.65	1.60	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interrelations of endocrine and physical growth variables and adolescents' psychological functioning are investigated. Participants are 56 girls and 52 boys, 9- to 14-years-old, and their parents. Participants are evaluated on biological and psychological variables at three times of measurement, six months apart. Biological measures include stage of pubertal development (Tanner stages), assessed by a physical examination, and hormone levels, assessed by blood samples for gonadotropins, gonadal steroids, adrenal androgens and cortisol. Psychological measures include assessments of psychological and behavior problems, cognitive functioning, self-esteem, affective states and interpersonal functioning. Assessment of parental behavior is through observations of parent-child interactions in standard laboratory situations and through standard inventories regarding childrearing attitudes and behavior. For boys, hormone levels were related to mother-reported aggression, specifically acting-out and rebellious behaviors. Adolescent self-reported anxiety and sad affect also appeared to play a role in the hormone-aggression relations. There were no relations between aggression and hormones for girls. The timing of changes in hormones was related to problems of adjustment in both boys and girls. Problems of confusion and sad affect were higher for boys with high-for-age adrenal androgens and for girls with high-for-age gonadotropins. The longitudinal data are being analyzed to examine how changes in hormone levels may influence the development of aggression, anxiety, and depression in young adolescents.

Project Description:

Interrelations of endocrine and physical growth changes and adolescents' cognitive, affective, and interpersonal competencies and dysfunctions are assessed. The objectives of this research are to examine, cross-sectionally and longitudinally: (a) the interrelations among these psychological processes in early adolescence (b) the relations between these psychological processes and biological variables (endocrine status and physical growth), and (c) the interrelations of childrearing variables, endocrine status, and psychological functioning in early adolescence. Areas of specific concern are: (a) biological variables and emotional characteristics that may be etiological factors in aggressive behavior, (b) effects of timing of pubertal endocrine and physical growth changes on problems of adjustment and peer relations, and (c) behavioral reactivity in stressful circumstances.

Methods and Findings:

Fifty-six boys (ages 10 to 14) and 52 girls (ages 9 to 14) and their parents are in the study. Plasma levels of gonadotropins (luteinizing hormone and follicle stimulating hormone), gonadal steroids (testosterone and estradiol), testosterone-estradiol binding globulin, adrenal androgens (dehydroepiandrosterone, dehydroepiandrosterone sulphate, and androstenedione) and cortisol, as well as height, weight, and stage of pubertal development are obtained. Psychological measures include: cognitive tests, daily self-ratings of moods, social support, aggression toward peers and academic achievement. Parents also report on their child-rearing attitudes and practices. Two measures aimed at identifying problem behaviors are a psychiatric interview (Diagnostic Interview for Screening Children) and parental assessment of behavior problems (Achenbach Child Behavior Checklist). The adolescents and their parents come to the laboratory where most of the measures are completed and where parent-adolescent interaction is observed while the family works together on conflict-resolution tasks (e.g., how families handle family disagreements regarding peer relationships of adolescents). The interactions are videotaped. The adolescents and one parent also are seen in the outpatient clinic where plasma is obtained for the hormone assays and the adolescents are examined for stage of pubertal development. During the clinic visit, the adolescents are observed for behavioral reactivity (e.g., fearful and anxious behaviors) while experiencing the potentially stressful phlebotomy procedure. These observational data and the cortisol data will be used to test hypotheses regarding stress, hypothalamic-pituitary-adrenal activity and behavioral reactivity in adolescents.

Findings indicate that levels of hormones that rise across puberty do relate to emotions and aggression in early adolescent boys, but not in girls. Boys who were higher on self-reported sad affect had a hormone profile of lower testosterone to estradiol ratio, lower testosterone-estradiol binding globulin, and higher androstenedione. Also, self-reported anxiety appeared to play a role in relations between hormones and aggression. Boys who were higher on acting-out behaviors were lower on anxiety. They also had a hormone profile of lower estradiol, testosterone to estradiol ratio, testosterone-estradiol binding globulin, and dehydroepiandrosterone sulphate as well as higher androstenedione. Further, boys who were higher on rebellious behavior (e.g. talks back, irritable, irresponsible) had a hormone profile of higher luteinizing

hormone and dehydroepiandrosterone and lower follicle stimulating hormone. Another question focused on whether the timing of changes in hormone levels is related to problems of adjustment. The hypothesis was that hormones will be related to behavior differently depending on whether levels are higher or lower compared to the adolescent's age-mates. Having a higher level relative to one's age-mates defined an earlier maturer while having a lower level relative to one's age-mates defined a later maturer. Problems of confusion and sad affect were higher for boys with high-for-age adrenal androgens and lower for boys with high-for-age sex steroids. Problems of confusion and sad affect were higher for girls with high-for-age gonadotropins.

Significance to Biomedical Research:

An increase in behavior problems and affective disorders during adolescence has been reported, but there are many questions regarding the etiologies of these problems. Findings from this study may help to clarify the interrelations among hypothalamic-pituitary-gonadal and adrenal axes activity during puberty, adolescent psychological characteristics, and parental childrearing practices.

Proposed Course:

Data collection is complete. Manuscripts are in press, submitted for publication, and in preparation. Additional cross-sectional analyses will be done for hormone relations to cognitive functioning, depression, and parent-child interaction. The longitudinal data on aggression, anxiety and depression and cortisol level and behavioral reactivity will be analyzed in the next year.

Publications:

Susman, E.J., Nottelmann, E.D., Inoff, G.E., Dorn, L.D., Cutler, G.B., Loriaux, D.L. and Chrousos, G.P.: The Relation of Relative Hormonal Levels and Physical Development and Social-Emotional Behavior in Young Adolescents. Journal of Youth and Adolescence. In press.

Hamburg, B.A. and Inoff, G.E.: Coping behaviors in Diabetes: Relationships Between Knowledge of Diabetes, Locus of Control, and Metabolic Control. In Ahmed, P. (Ed.): Living With Juvenile Diabetes. Springfield, Illinois, Charles C. Thomas, 1985, pp. 61-84.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02165-03 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adjustment in Early Adolescence: Family and Peer Influences

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Editha D. Nottelmann	Guest Researcher	LDP/NIMH
Other:	C. Jean Welsh	Research Psychologist	LDP/NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health

TOTAL ~~MAN YEARS~~ Person Years	PROFESSIONAL:	OTHER:
.30	.00	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Family and peer influences are investigated in the transitional period from childhood to adolescence. In early adolescence, children are at risk for adjustment problems, because they experience multiple change. Supportive relationships have been identified as moderators of stress for adults, but no data exist on the contribution of supportive relationships to psychological adjustment in early adolescence. Assessments focus on relations with family and peers.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

The study examines family and peer influences on psychological adjustment in early adolescence. Children are at risk for adjustment problems at this time, because they experience multiple change. They are making school and social transitions. They are entering puberty and beginning to take the first steps toward independence, moving away from their parents and establishing closer relationships with peers. Support from parents and peers is believed to buffer stresses generated by imposed change; and the lack of supportive relationships, therefore, is likely to heighten the risk for adjustment problems. Assessments focus on the contributions of family and peer relationships and peer-adult networks to early adolescent psychological adjustment.

Major Findings

As reported previously for the 162 adolescents (ages 12 to 13) who participated in the study, we found significant age and sex differences in their responses to questions about the quality of closeness of their relationships. We found a greater parent then peer orientation in the younger group (primarily among boys) and a greater peer than parent orientation among the older group (primarily among girls), a shift from parents to peers that seems to be related to physical maturity.

Significance for Biomedical Research

The factors contributing to, or protecting against, disordered affect and problem behavior in adolescence are poorly understood. This study is designed to provide information about the contributions of family and peer influences on children's psychological functioning that should be useful to mental health professionals.

Proposed Course

Several papers are planned. A manuscript on social networks in early adolescence is being submitted for publication.

This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02167-03 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interpersonal Inferential Abilities in Normal and Depressed Mother-Child Pairs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald J. Iannotti	Research Psychologist	LDP NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
	E. Mark Cummings	Senior Staff Fellow	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Affective Development

INSTITUTE AND LOCATION

NIMH, Building 15K, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205

TOTAL ~~MANYEARS~~ Person Years	PROFESSIONAL:	OTHER:
.80	.60	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Individual differences in abilities to interpret the internal states of other persons are examined in parents and their young children. How do mothers' abilities in this respect influence their functioning in relation to their children? Is the development of social inferential skills in the child related to mother's abilities and childrearing methods? Mothers' abilities to make appropriate inferences about internal states of others are measured using hypothetical situations involving interpersonal problem solving situations and through structured interactions with their children. Experimental situations involving distress or need in peers and adults are used to assess children's sensitivity to internal states. Mothers in the study differ in psychiatric diagnosis: major depression, minor depression, or normal.

Project Description:

The purpose of this research is to investigate mothers' abilities to make appropriate inferences about other persons' intentions, motives, and feelings and the relations of these skills to mothers' child rearing behaviors and to the development of inferential abilities in their young children. It is hypothesized that deficits in the parent may interfere with child-care functions and with the child's development of empathy and social sensitivity. It is also hypothesized that affective illness may systematically influence her inferential skills. The self-preoccupation and egocentrism sometimes observed in affectively ill adults may interfere with the ability to understand the experiences and needs of other persons, and thereby interfere with effective childrearing. Sometimes paradoxically, depression may have the opposite effect of heightened sensitivity to others' needs.

Methods:

Forty-eight mother-child pairs were studied. Mothers had DSM-III diagnoses of major depression, minor depression, or normal. The children were 2 to 2 1/2 years old. The mother's abilities to make inferences about others' psychological states were assessed in hypothetical social problem-solving situations and in structured interactions with the child. Children's abilities were assessed in both naturalistic and experimental situations.

Findings:

Mothers' childrearing methods were related to the social-inferential skills of their two-year-olds. Mothers of children who exhibited deficits in making inferences about the internal states of others directed their child's attention to his/her own behavior rather than to the behavior, thoughts, and feelings of peers. These mothers interacted disproportionately more frequently with the child's peer, when compared to mothers of children who demonstrated competence in social-inferential tasks. Mothers who directed their children's and their own attention to the behavior and feelings of others, encouraged positive social interaction, and did not use physical means to control their child's behavior when the child was two-years old, had children who performed well on social-cognitive tasks at five years of age.

There were no differences in the social-inferential skills of children, at two years and five years based on mothers' diagnoses. However, mothers who have experienced episodes of major depression differed from other mothers in their pattern of interacting with their child; they were generally less responsive to the child, were likely to direct the child's attention to others' feelings, were less controlling, used physical control and negative statements about the child's behavior less often, and were less likely to anticipate the child's needs or present future goals to the child.

Significance to Biomedical Research:

This aspect of development is important in the child's acquisition of adaptive skills in interpersonal relationships and in the child's understanding of his/

her social environment. It is important, therefore, to understand factors in the child's experience with the parent that facilitate or interfere with the child's interpersonal skills.

Proposed Course:

This is a final report. Manuscripts are being prepared for publication.

Publications:

Iannotti, R.J.: Naturalistic and structured assessments of prosocial behavior in preschool children: The influence of empathy and perspective taking. Dev. Psychol., 21: 46-55, 1, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02169-03 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions Between Siblings with a Depressed Parent

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Other:	Dale Hay	Guest Worker	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Child Behavior Disorders

INSTITUTE AND LOCATION

NIMH, Building 15K, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205

TOTAL ~~MAN-YEARS~~ Person Years:	PROFESSIONAL:	OTHER:
1.20	.35	.85

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Adaptive and maladaptive strategies of coping and defending learned in the parent-child relationship undoubtedly impact on the relationships between siblings. The aim of this research is to examine the links between mother-child interactions and sibling interactions in the domains of conflict and caregiving. The transmission of these patterns of behavior in families in which there is maternal depression is of special interest. Siblings (2-3 years old and 5-7 years old) are observed in interaction with each other, individually with the mother and together with the mother.

Project Description:

The adaptive and maladaptive coping strategies learned in the parent-child relationship undoubtedly impact on relationships between siblings. Although influences of siblings on one another have not had a tradition of theory to foster investigation (such as exists for the influences of parent on child), there is evidence of sibling effects on development. (For example, the highly aggressive child has been shown to be a significant teacher of aggression for his younger sibling.)

In the context of maternal depression, a number of hypothesized effects of possible deficits in mother's functioning might be expected to have consequences for sibling behavior. For example, when mother is unable to provide care, the older sibling may assume caregiver functions; mother's patterned styles of dealing with conflict, or of handling affect, may be incorporated into the repertoires of intersibling behavior. From another perspective, sibling relationships may be important in the context of parental depression. They may protect against or increase vulnerability to risks inherent in disturbed parental functioning. The purpose of the present study is to examine sibling interactions from both of these perspectives.

Methods employed and major findings:

In a laboratory setting designed to approximate the natural rearing environment (see Z01 MH 02144), siblings are observed in interaction with each other, individually with the mother, and together with the mother. The younger child is between the ages of two and three and the older child is between the ages of five and seven. In this study, unlike many other studies of parent-child interaction, the family members are not only observed while playing together, but while trying to accomplish other things: the mother provides a light meal, the older sibling watches over the younger while the mother is out of the room, the siblings have unequal resources, the children attempt to solve challenging and potentially frustrating problems, and the family reunites after the children have had separate stimulating experiences.

Two types of interactions are examined: (1) caregiving episodes in which one family member (mother, older sibling, or younger sibling) meets an explicit or implicit need of another, and (2) conflict episodes in which one family member protests, resists, or retaliates against another person's action. These types of episodes provide data on interpersonal competence and prosocial and aggressive interactions. The patterning as well as the content of caregiving and conflict episodes characterize each person's interactive style. Preliminary analyses indicate that in some instances older siblings "take over" the caregiving role of the depressed mother. More developmentally appropriate forms of caregiving of the younger sibling by the older sibling are seen in children with normal mothers. Particularly high levels of conflict are observed in some children with a depressed mother.

Significance to Biomedical Research and the Program of the Institute:

Intergenerational transmission of emotional disturbance may have both genetic and environmental components. Some forms of depression may be exacerbated or caused by deficits in social skills that are learned in the family setting and involve multiple channels of influence, including siblings.

Proposed Course:

Coding is in progress and will continue. About 25% of this work has been completed. Analytic plans are in preparation.

Publications:

Zahn-Waxler, C., Hollenbeck, B., and Radke-Yarrow, M. The origins of empathy and altruism. In M. Fox (Ed.) Annual Review of Animal Welfare Science and Philosophy. Martinus-Nijhoff, Publishers, The Netherlands, 1985, pp. 21-41.

Zahn-Waxler, C., Cummings, E. M. and Iannotti, R. (Eds.): Altruism and Aggression: Biological and Social Origins. Cambridge Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02170-03 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychiatric Evaluation of Infants and Toddlers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Leon Cytryn	Medical Officer (Psych)	LDP NIMH
Other:	Tracy Sherman	Senior Staff Fellow	LDP NIMH
	Donald H. McKnew	Medical Officer (Psych)	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Affective Development

INSTITUTE AND LOCATION

NIMH, Building 15K, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205

TOTAL ~~MANYEARS~~ Person Years:	PROFESSIONAL:	OTHER:
2.20	.90	1.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A psychiatric assessment procedure for children 1 1/2 to 5 years was developed and used in an ongoing study of offspring of normal and depressed parents. The procedure is a semi-structured play interview consisting of 3 10-minute segments: free play, doll play and aggressive play. The child's behavior is rated on a 15 item, 7 point unidirectional scale. A clinical rating is also made of overall psychiatric risk, emotional regulation, attachment, and coping and mastery. Finally, a clinical assessment is made of areas of concern in the child's behavior. The instrument was administered to 127 toddlers, and coding has been completed on 90.

These data have identified two groups of children who may be at risk for future psychopathology. One group shows repeated instances of maladaptive behavior throughout the observation session, while the other group shows less frequent, but more extreme forms of troublesome behavior. Follow-up research will indicate which of these patterns is most predictive of later psychopathology.

Project Description:

Affective illness in latency age children and adolescents has been given much attention. However, most studies have bypassed the toddlers, and reliable diagnostic instruments for use with young children have not been available. Although it may not be possible to make exact diagnoses at this young age, one would expect to find disturbances which might conceivably be precursors of future psychopathology. Findings of such precursors would contribute to a developmental view of affective illness and could be crucial to any attempts at secondary prevention.

A psychiatric assessment procedure for children 1 1/2 to 5 years is being developed and evaluated. The toddlers who are being evaluated are part of the NIMH Childrearing Project in which the children of 4 groups of mothers are being studied (major depression, bipolar disorder, minor depression, and normal) (see Project Z01 MH 02144).

Methods employed and major findings:

The procedure is a semi-structured play interview consisting of three 10-minute segments: free play with neutral toys, play with family toys, and aggressive play. Before the interview begins, separation from the mother is observed and noted. In each play segment, the child is encouraged to use the toys in any manner he or she chooses. Running notes are kept by the examiner and each session is recorded on videocassette. Following the session, the child's performance is rated on the mental status scale of the C.A.S.

In addition, the child's behavior in each of the 10-minute segments of the play interview is rated on 15 behavioral scales.

Following the behavioral assessment, a clinical rating is made of overall psychiatric risk, emotion regulation, attachment and coping and mastery skills. Finally, a clinical assessment is made of areas of concern in the child's behavior. Treatment recommendations are made. The instrument has been administered to 127 toddlers and coding has been completed on 90. Inter-rater reliability for the behavioral rating scales is 80%; for the clinical judgment scales, 85%. Using the behavioral ratings, a set of calculated assessments of the child's functioning in the areas of 1) Attachment, 2) Emotion regulation, 3) Coping and mastery, and 4) Overall risk for psychopathology is derived. A similar set of assessments is obtained from the clinical judgments. Overall risk scores ranged from 1(no risk) to 4(severe risk). Following are percentages of children falling into each risk category:

Behavioral rating (Calculated risk)	Clinical judgment (Clinical risk)
1 - 74%, 2 - 16%, 3 - 8%, 4 - 1.5%	1 - 48%, 2 - 20%, 3 - 14%, 4 - 13%

There is a high correlation between: calculated and clinical risk = .65, $p < .0001$; calculated and clinical emotional regulation = .39, $p < .005$; calculated and clinical attachment = .60, $p < .0001$; calculated and clinical coping and mastery = .56, $p < .0001$.

A comparison of the clinical rating of overall risk for the later development of psychopathology and the rating of overall risk calculated from the behavioral rating scales indicates that we have developed a reasonable model of the psychiatrist's behavior as he synthesizes his behavioral observations and reaches a summary judgment (The Behavioral Summary predicts 40% of the variance of the Clinical Summary). These two modes of evaluating the child's risk status yield two groups of children-at-risk. One group of children, identified by both the Behavioral Risk Measure and the Clinical Risk Measure, show multiple instances of maladaptive behavior. The second group of children are identified as being at risk only by the clinician's final judgment. These are children who do not show repeated worrisome behaviors. Rather, they have been identified as worrisome because of infrequent, but strikingly aberrant behavior. Follow-up research will indicate which of the patterns of behavior is most predictive of later psychopathology.

Significance to Biomedical Research:

The significance of this research is multifold: (1) The development of improved assessment instruments will help in understanding the developmental patterns of adaptation and maladaptation in very young children with limited language ability, and will permit more sensitive evaluation of the children's strengths and vulnerabilities. (2) These assessments will enable us to see whether patterns of adaptation differentiate between the children reared by normal and depressed mothers. (3) We will be able to evaluate how assessments from this perspective compare with assessments made from a set of standardized mother-child interactions. Patterns of adaptation and maladaptation observed in the child, while not satisfying conditions of a DSM-III diagnosis, may be indices of vulnerability to the later occurrence of problems. This prospective information not only adds to our understanding of the developmental course of affective illness, but may provide an informed basis for identifying children who are most at risk.

Proposed Course:

The remaining cases will be coded. A report for a scientific journal will be prepared.

Publications:

Sherman, T. & Asarnow, R.: The cognitive disabilities of the schizophrenic child. In M. Sigman (Eds.), Children with cognitive and emotional disorders: Assessment and treatment of dual disabilities. Orlando, Florida: Grune & Stratton, Inc., in press.

Cytryn, L., McKnew, D. H., Zahn-Waxler, C., Gershon, E. S.: Developmental issues in risk research: The offspring of affectively ill parents. In Rutter, M., Izard, C. E., Read, P. B. (Eds.): Depression in Children: Developmental Perspectives. New York, Academic Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 02171-02 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protective and Risk Factors in Childrearing: Contributions of Fathers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Elbert Wilson	Research Psychologist	DRG NIH
OTHER:	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS *(if any)*

Division of Research Grants
National Institute of Health

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.65	.55	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The research focuses on paternal contributions to child development. In particular, the role of fathers in families in which the mothers are depressed is examined. Families are selected for the study on the basis of the mother's diagnosis, as normal or depressed. Fathers, mothers, and two children (ages 2-5 and 8-11) are observed in interaction in a home-like environment established in the laboratory. They participate in a variety of planned situations representative of day-to-day family events. The family members are observed in dyads and triads as well as a total group. The father is also interviewed: a psychiatric assessment (SADS) and an interview concerning his involvement in childrearing. Data collection is in progress.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Reflecting as much the culture as biology, psychiatric and psychological theories of development have dealt almost exclusively with mothers as the determinants of the healthy or pathological development of offspring. The father's role has been ignored or has at best been considered in its absence. Even if pathology (e.g., depression) exists in the father, mothers' behavior remains the focus in offspring outcomes. For example, in such instances concurrent depression in the mother is often ascribed to "assortative mating"; thereby ignoring any dynamic influences of the father within the family affecting either the mother's or the child's mental health. In the present study, fathers' involvement in childrearing is the focus in families with and without maternal depression. The general research paradigm described in MH 02144 is utilized. The family (father, mother, two children ages 2-5 and 8-11) is brought into a home-like environment recreated in the laboratory; they are asked to participate in planned situations representative of typical day-to-day family events. Over a half day session, the family is observed in a variety of combinations: siblings alone, father alone with both children and alone with each child, and all family members together, including a family meal time. The father is interviewed about his involvement in childrearing and asked to describe an event with the children that captures for him the "essence of fathering." The father is also given a psychiatric assessment (SADS). These data provide information on fathers' interactions with children in relation to gender and age of child, and also on the father's rearing role when the mother is ill.

Forty families have been observed. Analyses focus on father interaction patterns: on interaction types, style, tone, purposes and amounts. Analyses are directed also to behavior patterns conceptualized in terms of relationships, namely, the relationship with his wife, the "climate" his presence creates. Father's reports on level of involvement with a variety of common childrearing tasks are examined. Of particular interest in all analyses is whether there are systematic differences between fathers in families with, and those without, a psychiatric diagnosis of mother's affective disorder.

Findings:

A preliminary analysis indicates that fathers with depressive spouses report significantly more involvement in child care and in meal preparation than fathers with well spouses. There is, however, no difference in their reported availability.

Significance to Biomedical Research:

This project provides data that will allow systematic study of the impact of the parenting unit on child behavior. Of particular interest is the behavior of fathers with wives suffering from affective disorders. The father's inclusion extends information regarding protective and risk factors in the child's rearing environment.

Proposed Course:

Data collection will continue. Coding is kept up-to-date with data collection. Analysis plans are underway. Another year of work is projected.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02172-02 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mothers as Mediators of Cognitive Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Sarah L. Friedman	Research Psychologist	LDP/NIMH
Other:	Malcolm A. Gordon	Research Psychologist	LDP/NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS: Person Years	PROFESSIONAL:	OTHER:
1.25	.75	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research is to investigate depressed mothers' influence on cognitive development of their children. It is hypothesized that a mother's depression interferes with her ability to serve as a mediator for her young child's cognitive development. Behavioral characteristics associated with depression might be expected to influence the mother's functioning in ways that would interfere with her ability to support the cognitive activity of her child. A woman's ability to maintain a shared attentional focus with her child, to respond adequately to the child's queries and to transmit information and thinking skills to her child is expected to be compromised when the woman is depressed. In this study these aspects of maternal behaviors are explored. Special emphasis is placed on the extent to which mothers impart facts and cognitive skills to their 2- to 3-year old children. These include facts about the physical, social and emotional worlds, methods for acquiring knowledge, methods for knowledge representation and for reasoning and problem solving. It is hypothesized that children whose mothers demonstrate low performance levels as cognitive mediators perform less well on I.Q. tests. The subjects are depressed and well mothers. I.Q. tests were administered to the children when they were between 5-7 years of age.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

The purpose of this research is to investigate depressed mothers' influence on the cognitive development of their children. It is hypothesized that a mother's depression interferes with her ability to serve as a mediator for her young child's cognitive development. Behavioral characteristics associated with depression might be expected to influence the mother's functioning in ways that would interfere with her ability to maintain a shared attentional focus with her child, to respond adequately to the child's queries and to transmit information and thinking skills to her child. The hypothesis that qualities of mothering influence children's cognitive growth is indirectly supported by two large bodies of research. Correlational research shows that even when the effect of genotype on I.Q. is experimentally partialed out, the environment in which children are reared has a significant effect on the level of their performance. Research about the effects of instructional programs on cognitive performance show that cognitive skills can be learned and applied by children who did not spontaneously use such skills. The objective of this project is to describe the specific mechanisms underlying environmental enhancement of and interference with children's mental development.

The role of the mother as a mediator of cognitive development is conceptualized in the following way: (a) The mother is responsible for creating and maintaining episodes of shared focus in which the child can learn from the mother; (b) The mother responds to her child's questions by providing cognitive content; (c) The mother through her action and speech provides the child with cognitive contents; (d) The mother motivates her child to engage in cognitive activity. The study explores these aspects of maternal behaviors, with special emphasis on the extent to which mothers impart cognitive contents. Cognitive contents were classified into four categories: (a) facts about the physical world, the social world and the emotional world; (b) methods for acquiring knowledge (e.g. asking questions, observing others, attending); (c) methods for knowledge representation (e.g., organizing referents in memory), (d) Methods for knowledge use (e.g., planning and reasoning).

The subjects are mothers and their 2- to 3-year-old children. Half of the mothers were diagnosed as depressed (major, unipolar depression according to DSM III classification) and half are well. Two types of mother-child interactions were selected from videotaped records (described in protocol 02144): (a) interactions during situations in which no teaching task was assigned to the mother and (b) interactions in which the mother has been given a specific teaching task. The children are seen again between the ages of 5 and 7, at which time they are administered the McCarthy Scales (an I.Q. test).

It is hypothesized that children whose mothers demonstrate lower performance levels in their role as mediators of cognitive development perform less well on cognitive tasks.

To date only the behavior of well mothers of 2- to 3-year olds has been analyzed. These mothers initiate about 50% of the episodes of shared focus. The majority of the cognitive contents imparted by these mothers

relate to (1) facts and principles and (2) methods of knowledge use, (e.g. reasoning, planning, problem solving). Less information is transmitted regarding methods for knowledge acquisition and knowledge representation. These latter findings are curious, since, theoretically, knowledge acquisition and representation are important skills that a toddler-age child must acquire. In close to 70% of the instances of shared focus mothers try to involve their children in some cognitive activity. When children query, mothers respond appropriately and with cognitive contents in 60% of the cases. Mothers do not introduce general referents and abstract concepts in talking to their toddlers; the referents of their talking are either present or occur within minutes prior to or following their speech. Mothers engage in minimal reinforcement of their children's engagement in cognitive activity such as decision making, reasoning or correct labeling of objects. Even when mothers are not asked to teach their children, their verbal and nonverbal behaviors are a rich source of information.

Significance to Biomedical Research:

The findings of this study will make it possible to isolate those aspects of the mother's behavior that interfere with or facilitate the child's cognitive development. Maternal depression may be associated with specific behaviors that are detrimental to children's acquisition of information, cognitive skills and motivation to engage in learning. If this is the case, the information can be utilized in prevention or remedial programs.

Proposed Course

Coding and analyzing of data will be completed. Depressed and well mothers will be compared. Also, the relation between mothers' behaviors when the children are toddlers and the children's performance three years later on sub-scales of a cognitive test (e.g. verbal, quantitative, memory) will be examined.

Publications

Friedman, S.L. and Cocking, R.R. Instructional Influences on Cognition and on the Brain. In Friedman, S.L., Klivington, K.A., and Peterson, (Eds.): The Brain, Cognition and Education. New York: Academic Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02173-02 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sex Identity Development in Young Offspring of Well and Depressed Mothers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tracy L. Sherman	Senior Staff Fellow	LDP/NIMH
Other:	Grazyna Kochanska	Guest Worker	LDP/NIMH
	Marian Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MANYEARS: Person Years	PROFESSIONAL:	OTHER:
.30	.15	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this investigation is to identify differences in maternal behavior that are associated with child's sex. Many studies have demonstrated differences between boys and girls in preferences for activities, self-concept development, attributional styles and patterns of cognitive development. Because, in adults, affective illness is 2 to 3 times more frequent in women than in men, we are examining how healthy and affectively ill mothers help their sons and daughters in handling social, emotional and cognitive challenges when the children are 2 to 3 1/2 years of age. These maternal behaviors will be examined in relation to assessments of child and mother when the child is between 5 and 6 years of age.

This study is a part of the Laboratory's Rearing Study (see Report #MH 02144) which provides extensive interactional data on mothers and their children in a naturalistic setting. By including in the design mothers differing in the quality and severity of affective disorder, it is possible to address directly the question of how maternal psychopathology is translated into childrearing behaviors that in turn provide the basis for the transmission of adaptive and maladaptive behavior patterns from one generation to the next.

Project Description

The goal of this investigation is to identify differences in maternal behavior that are associated with child's sex. Many studies have demonstrated differences between boys and girls in preferences for activities, self-concept development, attributional styles and patterns of cognitive development. Because, in adults, affective illness is 2 to 3 times more frequent in women then in men, we are examining how healthy and affectively ill mothers help their sons and daughters in handling social, emotional and cognitive challenges when the children are 2 to 3 1/2 years of age. These maternal behaviors will be examined in relation to assessments of child and mother when the child is between 5 and 6 years of age.

The data sources are videotaped observations of mothers and their 2-to 3 1/2-year-old children in a variety of naturalistic settings (see Project MH 02144). Similar observations are obtained two years later. In the follow-up observations, a series of gender-related probes have been incorporated which provide information about the mother's conscious preferences for her child as well as the child's own preferences for her/himself and knowledge of the cultural sex stereotypes. The study is not yet to a point where findings are available.

Significance to Biomedical Research

The successful development of one's own sex identity is crucial for the mental health of the individual. These data will provide information about adaptive and maladaptive methods for promoting the development of sex identity in young children. By including in the design mothers differing in the quality and severity of affective disorder, it will be possible to directly address the question of how maternal psychopathology is translated into rearing behaviors that in turn promote the transmission of adaptive or nonadaptive behavior patterns from generation to generation.

Proposed Course

The behavioral videotaped data from a sample of approximately 100 mothers during 3 half-day sessions have been collected. The gender-related probes for the follow-up study have been developed and are being used in the ongoing data collection. The coding instrument for assessing maternal behavior is currently being developed.

Publications

Sherman, T.: Categorization Skills in Infants. Child. Dev., In Press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02174-02 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Parental Beliefs Regarding the Origins of Their Children's Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Grazyna Kochanska	Guest Worker	LDP NIMH
Other:	Marian Radke-Yarrow	Chief	LDP NIMH
	Leon J. Kuczynski	Visiting Associate	LDP NIMH
	Sarah L. Friedman	Research Psychologist	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Affective Development

INSTITUTE AND LOCATION

NIMH, Building 15K, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.80	.50	.30

Person Years

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure of mothers' belief systems regarding the development of their children is investigated in families with and without parental depression. Mothers' perceptions of their own causal role in the development of their children as compared to other causal factors (genetics, father's input, external events), may be a crucial influence on their rearing practices. This issue becomes one of particular importance when clinically depressed mothers are concerned. Their possible feelings of helplessness regarding the development of their children, particularly their beliefs about their children's vulnerability to affective disorders, may influence their rearing behavior and the expectations conveyed to their children. Beliefs of 140 well and depressed mothers are assessed by means of a questionnaire and interview.

Project Description:

The structure of mothers' beliefs regarding the origins or determinants of their children's behavior is investigated in families with and without parental depression. It is assumed that parents' beliefs about themselves and their children affect how they function in the parental role (e.g., how they manage the child, the expectations placed on the child). Parental beliefs are especially relevant in a study of the environmental processes involved in the development of children whose parent(s) is(are) depressed. The depressed parent experiences helplessness, has distorted patterns of perceived control, and is self-deprecatory. Do these depression-derived characteristics in the parent translate into characteristics, beliefs, and behavior patterns in childrearing?

An instrument has been developed to measure the structure of the parent's causal thinking about the origins of specific dimensions of child behavior (affective, intellectual, social, moral qualities), along with the parent's assessment of the importance of each dimension and the child's status on each dimension. For each attribute the mother must weigh the causal contribution of her own behavior and personality, the child's father's behavior and personality, genetics, and external factors beyond her control.

The sample is 140 well and depressed mothers and their preschool and school-age children.

Findings:

Preliminary analyses suggest that affectively ill mothers are more critical of their children's development, particularly in the affective area, than are normal mothers. Also, they perceive differently the causes involved in child development. Mothers with major depression tend to place responsibility for child characteristics on the parents, while well mothers assign causal roles more evenly to parental and biological factors. Mothers with bipolar illness, on the other hand, emphasize the role of biological and other uncontrollable factors as determinants of child behavior. In general, the causal perceptions have different forms depending on the mothers' diagnoses. These differences will be examined in relation to mothers' interactions and relationships with their children and their children's behavior.

Significance to Biomedical Research:

Patterns of perceived personal control over life events have been found to be very different in normal and affectively ill groups. Depressed patients may manifest helplessness and experience lack of personal control over their own lives or may feel unduly responsible for their failures. These maladaptive patterns of perceived personal control may have consequences in the domain of their childrearing. Preventive measures and interventions will be better guided with an understanding of the interrelations of parental beliefs, their functioning with regard to their offspring, and their child's behavior.

Proposed Course:

Data collection will be continued to complete the sample. Analyses are underway. A manuscript is being prepared for publication.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02175-02 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Maternal Attributions Relevant to Child's Concept of Self

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marian Radke-Yarrow	Chief	LDP NIMH
OTHER:	Ruth Wylie	Guest Worker	LDP NIMH
	Barbara Hollenbeck	Social Science Analyst	LDP NIMH

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~MAN-YEARS~~ Person Years:	PROFESSIONAL:	OTHER:
.30	.20	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Knowledge concerning the young child's conceptions and feelings about the self and the origins of these evaluations is limited. In the present study these issues are explored. It is hypothesized that the early development of self-conceptions is heavily determined within the family. The mother's verbalizations to the child are investigated as a possible source. Since self-attributions of unworthiness are common in depressed adults, it seems possible that the depressed mother, in her comments to and about her child, may convey messages of the child's unworthiness or helplessness, the verbalizations of 18 well and 18 depressed mothers and their 2- to 3-year-old children in natural interaction are examined. Measures of the child from other data sources will be examined in relation to the mother's verbalizations.

Project Description:

The development of self-conceptions and evaluations is an important developmental task for young children. It is hypothesized that the early development of self-conceptions is heavily determined within the family. The verbalizations that mothers direct to or say about their children in their presence are investigated as a possible contributing influence. For children of depressed parents, self-evaluations are of special significance because self-attributions of hopelessness, helplessness, and unworthiness are common in depressed adults. There is the possibility that depressed parents convey such negative messages to and about their children, beginning when the children are very young. The cognitive and affective components of the verbalizations of well and depressed mothers and their 2- to 3-year-old children are studied.

Thirty-five minutes of their speech and behavior, in a range of rearing situations, have been videotaped. Written transcriptions have been made of everything that is said by mother, child, and any one present. Nonverbal behavior (e.g., facial expression, vocal tone, body movements) has also been recorded. For intensive study, 3 mothers' transcriptions are based on 5 hours of observed interaction. Mother's verbalizations are coded for the content of their attributions, the explicit or implicit nature of the messages and the occasions on which they occurred. The content of the child's verbalizations is also coded. Observations of the child in other settings will be examined in relation to mother's verbalizations.

Major Findings

Preliminary findings from a subsample of the cases identify a number of characteristics of mothers' attributions to their young children: Children are exposed to high frequencies of attributions; many more implicit than explicit. More attributions are rated positive evaluative than negative evaluative. However, when evaluations by the mother are strongly positive or negative, they tend to be explicit, and positives and negatives are similar in frequency. Most attributions are directed to specific aspects of the child rather than to the total person. Comments regarding child's competencies and cognitions occur with high frequency (about 35% of the attributions relative to other categories). Attributions about the child's feelings and emotions, lovability, and altruism each account for 1 to 3% of the attributions. Some aspects of the child's self (carefulness, self-control) receive negative comments almost exclusively.

Significance to Biomedical Research:

Self-evaluations constitute significant components of personality and enter into adaptive and maladaptive methods of coping. A better understanding of the origins of self-conceptions provides a significant element of knowledge.

Proposed Course:

Coding of mothers' input has been completed, analysis has begun. Coding of child data will continue. The coming year will be devoted to analyses.

A symposium presentation, Mothers' Attributions to their 2 1/2-year-old Children, was made by Ruth Wylie at an International Interdisciplinary Conference on Self and Identity, sponsored by the University College, Cardiff, Wales, July 1984. Manuscripts for publication will be prepared.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02207-02 LDP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Affective Rearing Environment: A Comparison of Normal and Depressed Parents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marian Radke-Yarrow	Chief	LDP NIMH
OTHER:	Leon Kuczynski	Visiting Associate	LDP NIMH
	Grazyna Kochanska	Guest Worker	LDP NIMH
	W. Elbert Wilson, Jr.	Research Psychologist	DRG NIH
	Barbara Hollenbeck	Social Science Analyst	LDP NIMH
	Judy Stilwell	Social Science Analyst	LDP NIMH

COOPERATING UNITS *(if any)*

Division of Research Grants, NIH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MANYEARS: Person Years	PROFESSIONAL:	OTHER:
1.75	.55	1.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The kinds and patterns of emotions expressed by parent and child, the circumstances and functions of these emotions, and their effects on the child are investigated in four studies that approach related issues. The objective of Study 1 is to obtain profiles of mother's and child's emotions in the normally occurring events of rearing. In Study 2, an experimentally-introduced mild stress is used to study the child's response to stress and mother's method of regulating the child's emotions. The regulation of children's affect in conditions of stress may be a particularly sensitive childrearing function that may be impaired by affective disorders in parents who have difficulty regulating their own emotions. The socialization of affection is examined in Study 3. Study 4 examines mother's patterns of responding to the child's emotions and the effects on the child. This process is also examined in the reverse: the child's handling of the mother's moods and emotions. Observations of mother and child over a range of rearing circumstances are the data source. Mothers with diagnoses of major depression, bipolar depression, or normal, toddlers (1-1/2- to 2-1/2-years-old) and school-age siblings (5- to 8-years-old) are the sample.

Project Description:

The objectives of these studies are to describe affective dimensions of rearing, and to examine the effects on children's emotional, social behavioral, and cognitive development of a complex of affective childrearing experiences. Four related studies focus on the kinds and patterns of emotions expressed by parent and child, the circumstances and functions of these emotions, and their effects on the participants.

Study 1. To characterize the affective contributions of mother and child to the rearing experience, each minute of 9 hours of observation was coded for the specific emotions expressed. This approach was adopted in order to obtain profiles of affect in continuing time, and to determine instigations to affect, handling of affect, and reciprocal effects of mother's and child's affects (Yarrow, Kuczynski, & Wilson).

Findings:

In a first analysis, total frequencies of emotions (i.e., number of minute interval in which they appeared) over three days of observations are summarized in terms of mothers' diagnostic groups. Anger, sadness, and low negative mood appeared, in ascending order of frequency, in well mothers, bipolar mothers, and mothers with major depression, respectively. Positive affect was expressed most by well mothers, least by manic-depressive mothers. Overt anxiety appeared most often in manic-depressive mothers.

At the level of group analysis, 2- to 3-year-old children closely match the affects of the mother; i.e., the diagnostic groups line up similarly on frequencies of each emotion. This is not the case with the school-age children with whom there is no distinct pattern for the three diagnostic groups.

Data for each of the three days observed indicate considerable consistency in sad and angry affect for the mother with major depression and less consistency for the other two groups. These analyses will be followed by examination of individual dyads in order to characterize dyadic relationships in affective terms, to determine who follows whom into an affective state, and to relate affective qualities to other facets of individual child behavior.

Epidemiological studies have described depressed mothers as showing little affection. Our data do not support this generalization. Frequency of affection is not different for the three diagnostic groups. However, a critical difference distinguishes the well from the depressed mothers. In the depressed group, affection is frequently expressed with sadness and anxiety. These double channels, we assume, convey a complex and possibly stressful message to the child.

Since depression in adults is more frequent in women than men, gender differences in early childhood are of interest. A suggestion of differences in rearing experience of girls and boys in affection between child and parent appears when initiations of affection are examined. Mothers initiate more affection to 2- to 3-year-old boys than to girls; however, girls of the same ages more often

initiate affection to mothers than do boys. A deficit discrepancy is suggested for little girls.

In Study 2 (Kuczynski & Yarrow) a situation of mild stress, related to the child's confrontation with a physical (anthropometric) examination, is being investigated for mother and child reciprocal influences.

Findings:

Severely depressed mothers manifest the most anxiety in this situation, and more frequently than other mothers react to their children with expressed negative affect and with more emotional content generally, thereby conveying anxiety to the child. Well mothers were more likely to cope with the situation by giving their child cognitive preparation for what is to be expected. The considerable individual differences will be examined in future analyses.

In Study 3, (Yarrow, Wilson & Kuczynski), physical contact and physical affection between parent and child are singled out for study. The kind of relationship between child and mother is, in part, reflected in these reciprocal interactions. The occasion for initiating contact, its positive or negative content, the kind of affection, and the reception of affection are being studied.

Findings:

Major analyses cannot yet be reported. A preliminary exploration suggests that very close and prolonged physical contact with the young child occurs with some severely depressed mothers.

In Study 4, (Yarrow & Kochanska) a primary objective is to learn how the child's emotions are handled by the mother and the effects of mother's behavior on the child's affective characteristics. The findings available begin to convey evidence. The mean length of negative emotional episodes (i.e., how long it takes for the child to return to emotional neutrality) is longest in the dyads with depressed mothers. The contributions of child and mother to this unregulated emotion will be studied. Another preliminary finding is that the ratio of positive to negative emotional dyadic engagements is 2 to 1 with well mothers and their children, but positive and negative engagements are of equal frequency in the two depressed groups.

Significance to Biomedical Research:

Affective experiences in early family relationships and specific affective characteristics of child and parent interaction are assumed to be significant indicators of, and contributors to, affective disturbance or well-being.

Proposed Course:

Studies are midway. Data collection, coding, and analysis will continue.

Publications:

Radke-Yarrow, M. and Sherman, T: Interaction of Cognition and Emotions in Development. In R. Hinde and A.N. Perret-Clermont (Eds.), Social Relationships and Cognitive Development, 1985, pp. 173-190.

Radke-Yarrow, M.: Affective Development in Young Children. In T. Berry Brazelton and M. W. Yogman (Eds.) Affective Development in Infancy. Norwood, N.J.: Ablex Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02209-02 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Attachment and Maternal Psychology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Mark Cummings	Staff Fellow	LDP/NIMH
Other:	Michael Chapman	Research Psychologist	Max-Planck Institute
	Leon Kuczynski	Visiting Associate	LDP/NIMH
	Marian Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS *(if any)*

Max Planck Institute, Berlin, West Germany

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MANYEARS PERSON YEARS	PROFESSIONAL:	OTHER:
.20	.15	.05

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [x] (a1) Minors
  - [x] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The quality of the child's emotional bond or attachment to the mother has been shown to be an important predictor of emotional and social functioning in early childhood. This research focuses on the nature of attachment relationships in children whose mothers are clinically depressed compared with children of normal mothers. Insecure and very insecure attachments were found to be significantly more frequent in offspring of parents with a major affective disorder, with the highest rate in children of bipolar parents.

Project Description:

The quality of the emotional bond or attachment the child forms to the mother appears to be one of the most important influences on general emotional and social functioning in early childhood, and a powerful predictor of later development. The mother's affective state and affective attributes are likely to play a role in the development of of attachments. The present research focuses on the relationship between the affective status of the mother and the quality of the child's attachment to mother. This research is also a first step toward emplically examining the role of disturbances in child-mother bonding in the transmission of depression.

Families in the Rearing Study (MH 02144) are the research participants. Fourteen offspring (15 to 47 months of age) of bipolar mothers, 42 offspring of mothers with major depression, 12 children of mothers with minor depressive disorders, and 31 children of mothers with no history of affective illness were studied. In general, children of mothers with a major affective disorder had a higher incidence of insecure attachment than controls but the bipolar group had an even higher occurrence of insecure attachment than the major depressive group. In addition, there was a significantly higher incidence of very insecure attachment in children of mothers with major affective disorders. Children of minor depressive mothers and normal mothers did not differ. Greater severity and prevalence of the mother's depressive episodes and more negative expressed emotion in the mother's rearing behavior over a nine hour span of observation also predicted insecure attachment.

Significance to Biomedical Research:

These data help to identify processes within families in which there is maternal depression. They show that, in addition to genetic risk, many of the young offspring of depressed parents experience conditions of rearing which, in themselves, are known to contribute to developmental problems, whether or not parental depression is present.

Proposed Course:

This is a final report.

Publications:

Radke-Yarrow, M., Cummings, E.M., Kuczynski, L., and Chapman, M.: Patterns of attachment in two- and three-year-olds in normal families and families with parental depression. Child Dev. 56: 591-615, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02210-02 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Symbolic Functioning in Play of Depressed and Well Mothers and Their Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Karen Caplovitz Barrett	Guest Researcher	LDP/NIMH
Other:	Sarah L. Friedman	Research Psychologist	LDP/NIMH
	Dennie Wolf	Research Psychologist	Harvard
	Malcolm Watson	Associate Professor	Brandeis

COOPERATING UNITS *(if any)*

Harvard University; Brandeis University

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL ~~MAN-YEARS~~ Person Years	PROFESSIONAL:	OTHER:
.50	.10	.40

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two parallel traditions of clinical work and developmental research have highlighted the importance of play as an indicator of adequacy of functioning. Children's play reveals their cognitive and affective status. In addition, mother-child play reveals the adequacy of functioning of that dyad. Play therefore is a particularly valuable context in which to study the effects of mothers' affective disorders on their children. The present study regards three related topics 1) the effects of maternal affective disorder (and chronicity of that disorder) upon symbolic complexity and affective aspects of children's play e.g., issues of nurturance or aggression; 2) the effects of maternal affective disorder on dyadic functioning in play -- for example, the extent to which she imposes her own agenda, and the ability of the dyad to sustain play; and 3) the relation between the dyadic variables and the child play variables, controlling for maternal diagnosis. Participants are 2-to 4-year-olds and their mothers who have been videotaped in semi-naturalistic conditions representative of early rearing experiences. Mothers have been diagnosed as normal or as depressed (bipolar, unipolar, minor depression).

Project Description:

The importance of children's play as an avenue for development and as a central activity revealing of the child's cognitive and affective status has long been recognized by researchers and clinicians. Play reveals capacities to sustain attention and interest in objects, as well as to use knowledge, affect, and imagination in a creative way. It is sensitive to developmental changes. The child's growing abilities to recreate in action events that occurred in the past, to conceive of certain objects or behaviors as adequate representations for other objects or behaviors, and to free its thinking from stimuli in the "here and now" are evidenced in the child's play. Play is also sensitive to concerns that the child has. It is a means through which the young child, whose verbal skill is limited, expresses what is on its mind. Play, it is assumed, makes possible wish fulfillment as well as repetition and gradual "working through" of traumatic or unassimilable experiences. The patterns of children's affect while they engage in different forms and levels of play have not been studied; in fact, researchers often appear to assume that play is accompanied by joy and excitement.

It is hypothesized that a child's experiences with an affectively disordered mother should influence both cognitive and affective features of the child's play. It is also predicted that mood disorders will interfere with the mother's ability to become involved with the child in play.

This study will test these hypotheses by examining the narrative themes, the affect-relevant themes (e.g. aggression, fear, helplessness, nurturance, mastery), the cognitive level (sensorimotor, presymbolic, symbolic), the veridical affect during the play of the children, and other more specific characteristics of the play. The mother's activity with the child in play also will be assessed: the supportive or interfering nature of the mother's interventions, the strategies used to communicate the borderline between fantasy and reality, and how much departure from reality is allowed or encouraged.

The research participants are 2- to 4-year-olds and their mothers, who have been seen in semi-naturalistic conditions representative of early rearing experiences (see MH 02144). Mothers have been diagnosed as normal or as depressed (bipolar, unipolar, minor depression).

Data for the present study are coded from videotapes. A comprehensive coding system for scoring the videotapes has been developed. This includes assessment of maternal involvement, directionality and hedonic valence of exchange between mother and child in play, and the narrative themes of pretense play and the cognitive levels of the mother's and the child's play. The tapes are coded by researchers who are blind to maternal diagnosis.

Significance to Biomedical Research:

This study concerns the impact of maternal depression both on central aspects of young children's development, and on crucial features of mother-child dyadic exchange. Findings will have important implications for clinicians and parents concerned with 1) how maternal depression impacts the young child, 2) how maternal depression impacts on mother's ability to communicate and negotiate with her child and 3) how the behaviors of depressed (and well) mothers affect their children's cognitive and affective status.

Proposed Course:

Behavior has been coded for an initial study, regarding the influence of maternal involvement and affective disorder on symbolic functioning and duration of focused attention in play. Analysis is underway and a manuscript is projected for completion in 3 to 4 months. Coding for affective aspects of play are projected for completion in the coming year.

Publications

Bertenthal, B., Campos, D., and Barrett, K.C.: Self-produced Locomotion: An Organizer of Emotional, Cognitive, and Social Development in Infancy. In Emde, R, and Harmon, R. (Eds.): Continuities and Discontinuities in Development. New York, Plenum, 1984, pp.175-210.

Caplovitz, K.D., and Campos, J.: Emotion and Self. In Harre, R. and Lamb, R. (Eds.): Encyclopedic Dictionary of Psychology. Oxford, England, Basil Blackwell Publishers, Inc., in press.

Campos, J., Emde, R., and Caplovitz, K.: Emotional Development. In Harre, R. and Lamb, R. (Eds.): Encyclopedic Dictionary of Psychology. Oxford, England, Basil Blackwell Publishers, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02229-01 LDP

PERIOD COVERED

October 1, 1984 through September 31, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Vocalic Analysis of Natural Discourse in Well and Depressed Mothers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Zvia Breznitz	Visiting Associate	LDP NIMH
Other:	Tracy L. Sherman	Senior Staff Fellow	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20205

TOTAL ~~MAN-YEARS~~ Person Years	PROFESSIONAL:	OTHER:
.20	.20	.00

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study examined the speech behavior of well and depressed mothers during conversation with their 3-year-old children. The sample to date consists of 18 well and 10 depressed mothers. Given the general motor retardation, the reduced energy level and social withdrawal of depressed individuals, it was predicted that the speech patterns of depressed mothers would differ from the speech patterns of well mothers. It was found that depressed mothers vocalize less often than do healthy mothers. The children of the depressed mothers also show differences in their speech patterns when compared to the children of healthy women. They speak less to their mother while sitting and eating lunch with her. While waiting with their children for a doctor's visit the depressed women changed their speech pattern to one of increased production punctuated by many very brief pauses. Similarly, their children's speech increased in amount to more closely approximate that of normal children. This change does not occur in the speech of the children of healthy mothers.

The overall pattern of behavior suggests that depressed mothers interact differently with their children than do healthy mothers. This may affect the quality of mother-child interaction. It is hypothesized that the reduced frequency of vocalizations by depressed mothers may reduce the reinforcing and socializing capacity of speech for her children. The differences in the behavior between the two groups of children indicate that these influences are already apparent and affecting the behavior of her three-year-old offspring.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

The purpose of this study was to examine various features in speech behavior of well and depressed mothers during conversation with their children. Given the general motor retardation of depressed persons, their reduced energy level and their social withdrawal, one would hypothesize that their speech patterns differ from those of well persons. It is conceivable that depressed mothers speak less, have longer pauses and have longer latencies in responding to speech initated by their children. If the speech patterns of depressed mothers are distinctly unique, this may affect the mother-child interaction in meaningful ways. Thus, it is conceivable that long pauses and long latencies discourage children from verbal interaction and reduce some of the reinforcing capability of speech.

The sample to date is 18 well and 10 depressed mothers and their 3-year-old children. Two studies examined the non-content aspect of speech behavior in the mother-child interaction: The first study focused on the analysis of temporal patterns of the dialogues. The central parameters used to analyze the sound pattern of conversation were: pauses, vocalizations, speaking turns, switching pauses, and simultaneous speech.

The second study focused on voice characteristics of the speakers with particular emphasis on the emotional features of voice: voice pitch, pitch range, pitch variability and loudness.

The conversations between the dyads were taken from audio-video tapes of interaction in the home-simulated environment of the NIMH Child Rearing Study. The total sum of recorded conversation for each case was 15 minutes.

In Study 1 data from conversations between well and depressed mothers and their children have been collected and analyzed. The main significant results are that:

1) The total duration of the mother's speech to her child was less for depressed women than well women in the relaxed situation of their preparing lunch.

2) In the period of stress in which mother and child are together awaiting a visit by a doctor, the pattern of speech in the dyads with depressed mothers changes from the pattern shown while the mother was preparing lunch in a manner opposite to that of the normal mothers. The depressed mothers change to speaking more and to having more frequent pauses in their speech, while the normal mothers change to speaking less with fewer pauses.

3) When in a relaxed situation, seated together eating lunch, children of depressed mothers speak significantly less than do children of normal mothers. However, in the period while waiting for the doctor, these differences disappear. The small stress created by waiting for the doctor has a dramatic effect on the children of depressed mothers, causing the amount and frequency of their speech to appear similar to that of the normals. The change in the pattern of speech observed in the depressed women and their children is referred to clinically as one that is indicative of high levels of anxiety. The normal mothers and

their children did not show this pattern.

Study 2 -- Data are being collected.

Significance to Biomedical Research

Dialogue between mother and child is a basic process by which children are socialized. This research has demonstrated that fundamental aspects of the mother's speech are disrupted when she is depressed. She speaks less frequently than normal mothers, when she speaks she speaks more slowly, and critically, her speech is less contingent on her child's speech. The depressed mother would appear to be, therefore, a less effective teacher and socializer.

Proposed Course:

A report on these findings (Study 1) is currently being written.

The data for Study 2 are currently being collected.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02230-01 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychiatric Assessment of Toddlers Based on Observation of Mother-Child Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Donald H. McKnew	Medical Officer (Psych)	LDP NIMH
Other:	Leon Cytryn	Medical Officer (Psych)	LDP NIMH
	Tracy Sherman	Senior Staff Fellow	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Affective Development

INSTITUTE AND LOCATION

NIMH, Building 15K, NIH, 9000 Rockville Pike, Bethesda, Maryland 20205

TOTAL ~~MAN YEARS~~ Person Years	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project brings together the strengths of clinical psychiatry and developmental psychology in developing two means of assessing adaptation and maladaptation in toddlers. The child and his/her mother are observed in interaction in six situations which include variations in stress, relaxation, and pleasure (see Project Z01 MH 02144). To date, 90 child-mother dyads have been scored. The clinical judgment of the child's risk for the later development of psychopathology and the behavioral rating of the child's risk correlate at the level of .79, $p < .001$. What is indicated by this finding is: We now have a reasonable model of the clinician's functioning as he synthesizes his observation of the child's behavior; 64% of the variance in the clinician's judgment is accounted for via the summarization of the behavioral observation. The behavioral observation system is more conservative in giving high or moderate risk scores to the child in that it requires an overall pattern of poor functioning (12% of the children received risk scores of moderate or severe). In addition to these children, the clinician's judgment also gives high or moderate risk status to children who may show infrequent, but severe patterns of problematic behavior (21% of the children). Follow-up research will indicate which of these patterns of vulnerability is most predictive of later psychiatric problems.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

In the present project, we are attempting to bring together strengths of the clinician and the child developmentalist. The characterizations of child behavior in developmental and clinical research generally reflect the concepts and tools of the specific disciplines. The clinician's sources of information about the child tend to be the clinical interview and/or the child's presenting problems, but rarely is systematically observed behavior the data source. The developmental psychologist relies on structured questioning of the mother and on direct obeservation of the child. Child behavior is usually cast in terms of specific behaviors (smiling, attending, complying, etc.) occurring in sequence. These frequencies and chains are then recast into conceptualizations of child functioning.

In the current project an analysis scheme has been developed which draws upon the approaches of the two disciplines. Interaction of child with mother is observed in situations that reveal the child's coping skills, mood regulation, and attachment behaviors. Specific stresses that are natural parts of the child's life such as waiting for lunch when hungry, attempting to solve frustrating problems, coping with the mother's unavailability while she attempts to nap, meeting a stranger with mother present, as well as situations of pleasure and relaxation (see Z01 MH 02144).

The behavior is coded by experienced clinicians: Each episode is scored first for the presence or absence of specific behaviors. Then clinical judgments are made on eight scales: level of motor activity, content of thought, social attentiveness, social responsiveness, social initiation, coping and mastery, and emotional expression and regulation. The behavior is not scored against a presumed age norm (since age norms do not exist for these behaviors). From these scores, a summed score across situations is obtained. A clinical assessment (areas of concern, defenses or coping strategies, assessment of child's risk status for later development of psychopathology, diagnosis, and treatment recommendations) is also made after viewing and rating the six interaction situations.

The toddlers who are being evaluated are part of the childrearing project in which children of four groups of mothers are being studied (major depression, bipolar disorder, minor depressions, and normal) (see project Z01 MH 02144).

Ninety cases have been coded. Inter-rater reliability for the presence or absence of specific behaviors is 85%; for the clinical judgments for the scales, approximately 90%.

Using the behavioral rating, one derives a set of calculated assessments of the child's functioning in the areas of attachment, emotional regulation, coping and mastery, and level of motor activity. An overall calculated risk score is derived by using specific combinations of these behavioral scores. A similar assessment of attachment, emotional regulation, and risk is derived from the clinical report made after completing the behavioral ratings.

Risk scores for both methods of assessment are made from 1(no risk) to 4 (severe risk). Following are the percentage of children falling into each risk category:

Behavior rating (Calculated Risk)	Clinical Judgment (Clinical Risk)
1 - 57%, 2 - 32%, 3 - 8%, 4 - 4%	1 - 49%, 2 - 30%, 3 - 18%, 4 - 3%

There is a high correlation between: calculated and clinical risk = .79, $p < .0001$; calculated and clinical attachment = .63, $p < .0001$; calculated and clinical emotional regulation = .50, $p < .0001$.

Significance to Biomedical Research:

An assessment instrument has been developed that will help us to understand adaptation and maladaptation in toddlers, will permit more sensitive evaluation of the child's strengths and vulnerabilities, and will permit comparisons of patterns of adaptation of the children reared by normal and depressed mothers. These assessments of patterns of adaptation and maladaptation in the young child may be indices of vulnerability to the later occurrence of psychopathology. This prospective information will add to our understanding the developmental course of affective illness, and provide an informed basis for identifying children who are most at risk.

Proposed course:

Coding will be completed and a manuscript will be prepared within the next year.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02231-01 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological-Behavioral Relations in Early Adolescence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Editha D. Nottelmann	Guest Researcher	LDP/NIMH
Other:	Elizabeth J. Susman	Senior Staff Fellow	LDP/NIMH
	Gale E. Inoff	Research Psychologist	LDP/NIMH
	Lorah D. Dorn	Social Science Analyst	LDP/NIMH
	George P. Chrousos	Senior Investigator	DEB/NICHD
	Gordon B. Cutler, Jr.	Senior Investigator	DEB/NICHD
	D. Lynn Loriaux	Chief	DEB/NICHD

COOPERATING UNITS *(if any)*

Laboratory of Developmental Endocrinology Branch, National Institute of Child Health and Human Development

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MANYEARS: Person Years	PROFESSIONAL:	OTHER:
1.10	.80	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interrelations of endocrine, pubertal development, and physical growth variables and adolescent adjustment are investigated. Participants are 9- to 14-year-old boys and girls and their parents. The adolescents and their parents are seen three times, six months apart. Biological measures include plasma hormone level (gonadotropins, sex steroids, and adrenal androgens), stage of pubertal development (Tanner criterion), and height and weight. Psychological measures include assessment of psychological adjustment and behavior problems, cognitive functioning, competence, and self-esteem. Cross-sectional analyses (based on data from the first time of measurement) examining relations among biological measures show sex steroids to be the strongest hormonal correlates of pubertal development and physical growth in boys and adrenal androgens to be the strongest hormonal correlates of pubertal development and physical growth in girls. Cross-sectional analyses of biological and psychological measures revealed more biological correlates of adolescent adjustment and behavior problems for boys than for girls. They also revealed that asynchrony between developmental indices had implications for adjustment and behavior problems. Longitudinal analyses will examine stability and change in adolescent adjustment and behavior in relation to changes in pubertal and endocrine status.

Project Description

Interrelations of endocrine, pubertal stage, and physical growth indices of pubertal development and adolescent adjustment and behavior are investigated. The objective of the study is to examine cross-sectionally and longitudinally interrelations among maturational processes and psychological adjustment and functioning in early adolescence.

Methods and Findings

The participants are 108 adolescents (56 boys, 52 girls), between 9 and 14 years of age, and their parents. Biological measures include plasma hormone level (gonadotropins: luteinizing hormone (LH), follicle stimulating hormone (FSH); sex steroids: testosterone (T), estradiol (E_2); and adrenal androgens: dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione), stage of pubertal development (Tanner criterion), height, and weight. Psychological measures include assessments of psychological functioning (self-image, competence, self-esteem), cognitive functioning, interpersonal functioning, and behavior problems.

In general, plasma hormone levels were higher at each successive pubertal stage. The cross-sectional results (Time 1), with different boys and girls at each pubertal stage, suggest that from Stage 1 to Stage 5 there is a two-to threefold increase in gonadotropin levels; that testosterone levels increase 18-fold in boys and twofold in girls; that estradiol levels increase twofold in boys and eightfold in girls; that, in boys, DHEA and androstenedione levels increase twofold, with no discernible trend for DHEAS; and that, in girls, DHEA and DHEAS levels increase twofold and androstenedione levels increase threefold.

Cross-sectional analyses of interrelations among measures of hormone level, pubertal stage, and physical growth indicate that for both boys and girls, as a group, physical maturation is an orderly, age-related developmental process. Sex steroid (testosterone) level was the strongest hormonal correlate of pubertal development and physical growth for boys; and adrenal androgen (androstenedione) level was the strongest hormonal correlate of pubertal development for girls, except for menarchial status, which was most strongly related to estradiol.

Analyses of relations between biological and psychological measures were done to examine whether early adolescent adjustment and behavior vary with hormone levels per se or with hormone levels that are low or high for age or for pubertal stage. The cross-sectional results indicate that hormone-behavior relations generally are stronger and more consistent for boys than for girls.

Behavior problems (mother ratings) were related to hormone levels per se: boys with a profile of lower sex steroid and higher adrenal androgen levels had higher scores for obsessive-compulsive (testosterone androstenedione) and delinquent (estradiol, androstenedione) behaviors; and girls with lower adrenal androgen levels (DHEAS) had higher scores for depressive withdrawal and delinquent behaviors.

The moods of boys (adolescent and mother ratings) were related either to low hormone levels or to hormone level for pubertal stage. Positive moods were associated with either lower sex steroid or lower adrenal androgen levels or with low levels of these hormones at higher pubertal stages.

Adjustment problems (adolescent self-ratings) were related most strongly to hormone level for age. For example, older boys with a profile of relatively low sex steroid (T/E_2 ratio) and high adrenal androgen (androstenedione) levels had more problems with emotions, social relationships, future orientation, as well as general coping problems. Higher levels of adrenal androgens (androstenedione) in older boys at earlier stages of pubertal development also were associated with social adjustment problems, i.e., with lower prosocial scores.

Competence (adolescent self-ratings) generally was associated with low hormone levels in boys and girls who were early maturers (with a profile of lower age at higher pubertal stages): in both boys and girls, cognitive competence was associated with relatively low gonadotropin (FSH) levels; additionally, in boys, social competence and general competence (or self-esteem) were associated with relatively low adrenal androgen (androstenedione) levels; additionally, in girls, social competence was associated with relatively low gonadotropin (LH) levels, and physical competence was associated with relatively low sex steroid (E_2) levels.

Significance to Biomedical Research

The increase in adjustment and behavior problems during adolescence has been documented extensively. Findings from this study begin to clarify how such problems are related to pubertal status (i.e., hormone level, pubertal stage, and physical growth) in early adolescence. The findings also are of immediate value to developmental endocrinologists (hormone-pubertal stage and hormone-physical growth relations, as well as hormone-behavior relations provide normative data that can be used to evaluate clinical cases of endocrine abnormalities) and also to pediatricians, adolescent medicine specialists, and child psychiatrists. The findings have implications for prevention and for intervention programs.

Proposed Course

Additional cross-sectional data analyses are planned for preparation of manuscripts on (a) maturational correlates of competence and self-esteem, (b) relations between social cognition and pubertal development, and (c) psychological profiles of early adolescent boys and girls.

Processing of longitudinal data (Time 2 and Time 3) continues. Longitudinal analyses will focus on three important issues in early adolescent development: (1) interrelations of changes in hormone level, pubertal development, and physical growth; (2) stability and change in adolescent adjustment in relation to change in maturational status (hormone level, pubertal stage, and physical growth); and (3) the effect of rate of physical maturation (rate of progression through pubertal stages) on adolescent adjustment.

Publications

Nottelmann, E.D., Susman, E.J., Blue, J.H., Inoff, G.E., Dorn, L.D., Cutler, G.B., Jr., Loriaux, D.L., & Chrousos, G.P. Gonadal and adrenal correlates of adolescent adjustment. In Lerner, R.M., and Foch, T.L. (Eds.): Biological and psychosocial interactions in early adolescence: A life-span perspective. Hillsdale, N.J., Erlbaum, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02232-01 LDP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Development of Attention Span in Children of Depressed and Well Mothers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Zvia Breznitz	Guest Researcher	LDP/NIMH
Other:	Sarah L. Friedman	Research Psychologist	LDP/NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL MAN-YEARS: Person Years	PROFESSIONAL:	OTHER:
.25	.15	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this study is to investigate (a) the development of toddlers' attention span in children of depressed and well mothers; (b) maternal influences on the development of attention and (c) the effects of depression on mothers' ability to regulate their young children's attention span. There is very little information about the development of attention in the transition years between infancy and early childhood. In addition, the role of mother as a regulator of a child's attention is not well understood and calls for detailed investigation. Attention, like many other skills may need to be at least partially trained. Mothers, through their interaction with their children, are hypothesized to provide such training. They are hypothesized to recruit their children's attention and to maintain it through their involvement, feedback regarding the children's activity and through reinforcement. Since depression entails a diminished ability to concentrate, loss of interest in usual activities and irritability, depressed mothers are hypothesized to be less likely than well mothers to train their children's attention. Consequently, children of depressed mothers are hypothesized to have deficits in attention. 29 depressed women (unipolar depression) and 29 well women were observed with their children. The children were between 16 and 44 months at the initial observation and some were already observed 3 years later. Preliminary analyses indicate that toddlers of depressed women have a shorter attention span.

Project Description

The objective of this study is to investigate (a) the development of toddlers' attention span in children of depressed and well mothers; (b) maternal influences on the development of attention and (c) the effects of depression on mothers' ability to regulate their young children's attention span.

The centrality of attention for human functioning is unquestionable. Studies of attention in childhood have focused on the period of infancy and on school age. There is very little information about the development of attention in the transition years between infancy and early childhood. In addition, the role of mother as a regulator of a child's attention is not well understood and calls for detailed investigation. Attention, like many other skills, may need to be at least partially trained. Mothers, through their interaction with their children, are hypothesized to provide such training. They are hypothesized to recruit their children's attention and to maintain it through their involvement, feedback regarding the children's activity, and through reinforcement. Since depression entails a diminished ability to concentrate, loss of interest in usual activities and irritability, a depressed woman is less likely than a well mother to recruit and work on maintaining her child's attention. Consequently, children of depressed mothers are hypothesized to have deficits in attention. The effects of a mother's depression on her child's attention are expected to be cumulative. As children grow older, they face tasks that are more complex and that are demanding of more attention. Children's early attention deficits may become more severe as children are faced with such tasks and as they find themselves unable to recruit well-developed attentional capabilities.

29 mothers diagnosed as depressed and 29 well mothers were observed while with their children. For details see protocol Z01 MH 02144. They were first seen when their children were between 16 and 37 months of age and again about 3 years later. Additional data about the school performance of the children will become available. The videotapes of mother-child interaction were analyzed to provide data regarding the three research objectives outlined above. Preliminary analyses show that during the 15 minutes in which the mothers and toddlers were observed, the children of the depressed women attended to a significantly larger number of objects ($p < .01$).

Significance to Biomedical Research

The study will highlight developmental aspects and environmental influences on attention. Some studies of children of depressed women have shown that these children have attention deficits. Yet, the processes underlying these deficits are currently not known. The present study is a preliminary attempt to understand such processes.

Proposed Course

The data will be further analyzed to answer each of the three research questions indicated above.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00478-29 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neural mechanisms of memory and habit formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Mishkin	Chief	LN NIMH
Others:	E.A. Murray	Senior Staff Fellow	LN NIMH
	J. Bachevalier	Visiting Associate	LN NIMH
	D. Kowalska	Visiting Associate	LN NIMH
	H. Petri	Chairman	Towson State Univ.
	W. Overman	Asst. Professor	Univ. of North Carolina

COOPERATING UNITS *(if any)*

Towson State University
University of North Carolina

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	3.0	3.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The anterior temporo-insular cortex in the macaque consists of the highest-order sensory processing stations for all the sensory modalities. We have proposed that this cortex contains the stored representations of stimuli to which the organism has been exposed. The storage is presumed to be the result of activation by anterior temporo-insular neurons of a limbo-thalamo-cortical pathway, which actually consists of two parallel pathways, one involving the amygdala, the magnocellular portion of nucleus medialis dorsalis, and orbital frontal cortex, and the other involving the hippocampus, anterior thalamic nulcei, and the cingulate cortex. Recognition memory occurs when the stored representation of a stimulus is reactivated by the same stimulus acting currently, and associative memory occurs when that stored representation activates, or is activated by, the stored representation of another stimulus or another event, such as a location, an emotion, or a motor act. Evidence has been obtained suggesting that the amygdaloid circuit is selectively involved in the first of these functions (i.e. stimulus-stimulus associations), whereas the hippocampal circuit is selectively involved in the second (i.e. stimulus-location associations). All of these forms of memory can be distinguished from habits, which appear to be independent of the limbo-thalamic system and which may depend instead on the cortico-striatal system.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION:

The objective of the studies in this project is to delineate the neural system underlying memory formation in the monkey and to differentiate it from the neural system that underlies habit formation. The methods used include behavioral analyses of the effects of selective cerebral ablations and disconnections combined with anatomical analyses of functional neural pathways. The rationale and design of the studies are often based directly on information derived from other projects in this laboratory, many of which deal with the pathways for, and mechanisms of, stimulus processing and encoding. The results from these and other projects suggest that the sensory system for each modality is composed to two hierarchically organized corticocortical pathways, one directed ventrally to the temporal-lobe limbic system and concerned with object perception, and the other directed dorsally to the frontal-lobe motor system and concerned with spatial perception. The ultimate goal of this project is to determine how object and spatial perceptions in the different modalities are formed into memories, how these different memories are associated with each other, how they evoke emotions and motor acts, and how they lead not only to these cognitive events but also to habit formation. Our progress in understanding each of these processes will be described in turn.

(1) Recognition memory

Previous work has indicated that visual recognition memory (assessed by delayed nonmatching-to-sample with trial-unique objects) is mediated by a cortico-limbic-thalamic system composed of two largely separate circuits arranged in parallel. One of these circuits consists of the amygdala, amygdalofugal pathways, and the magnocellular portion of the medial dorsal nucleus (MDmc), and the other, the hippocampus, fornix, and anterior nuclei of the thalamus (Ant N.). The evidence for this conclusion is that damage to the amygdaloid and hippocampal circuits at each stage in the system (i.e. medial temporal lobe, limbothalamic pathways, or medial thalamus) causes a severe loss in recognition memory, but only when the two circuits are damaged in combination, separate damage leading at most to only mild deficits. More recently we have examined the effects of ablating the frontal cortical projection zones of MDmc and Ant N. and found again that recognition failure followed combined but not separate ablation of structures related to the amygdaloid and hippocampal circuits, in this case the ventral and medial prefrontal cortex, respectively. Ablation of prefrontal cortical regions outside the projection zones of MDmc and Ant N., by contrast, produced little impairment. These results indicate that the ventromedial region of the prefrontal cortex constitutes still another stage in the limbic memory system.

Although all of our evidence to date supports the conclusion that combined damage to the amygdaloid and hippocampal circuits is necessary to produce loss of visual recognition, it is not yet certain that within the temporal lobe itself damage to the amygdala and hippocampus alone is sufficient to yield this effect. The tissue whose role is in question is the rhinal cortex, i.e. the complex made up of peri-, pro-, and ento-rhinal cortex, which relays information from the sensory processing pathways into both the hippocampus and amygdala and, independently, into MDmc. To study the role of this tissue in

memory, we have been examining the effects of ablating (a) the amygdala and rhinal cortex but not the hippocampus, (b) the hippocampus and rhinal cortex but not the amygdala, and (c) the amygdala and hippocampus but not the rhinal cortex. The results have been obtained for combinations (a) and (b), and they indicate that, whereas adding rhinal lesions to hippocampectomy yields little additional impairment, adding rhinal lesions to amygdalectomy produces an impairment equal in severity to that of removing all three structures in combination. From this it appears that the rhinal cortical ablation essentially deafferents the hippocampus, thereby rendering it nonfunctional and so mimicking its removal. By contrast, the rhinal cortical ablation clearly does not deafferent either the amygdala or MDmc, and indeed it is known that, unlike the hippocampus, the amygdala receives inputs directly from the sensory processing pathways, inputs which it can then relay further to MDmc. Because the results are not yet available from lesion combination (c), it is still unknown whether, in the absence of the amygdala and hippocampus, the rhinal cortex provides MDmc with sufficient input from the sensory processing pathways to sustain recognition memory to any significant extent. It is therefore not yet clear whether the rhinal cortex constitutes a third medial temporal-lobe component of the limbic memory system.

While most of our efforts in the past have focused on visual object recognition, we have also been engaged in studies of recognition memory in other modalities. The studies in the somatosensory modality have progressed the farthest; these have demonstrated that combined but not separate amygdaloid and hippocampal ablations cause object recognition failure in touch as well as in vision. Unlike the pathway in vision, however, the sensory processing pathway through which tactual information reaches the medial temporal limbic structures has not yet been established. Evidence from another project (MH-02037) suggests that the most likely pathway is one that proceeds from the primary and secondary somatosensory areas through the insula, a possibility we are currently testing by examining the effects of insular lesions on tactual recognition.

Unlike visual and tactual recognition, auditory recognition has proven extremely difficult to demonstrate in monkeys. After several failed attempts, however, we believe that we now have a testing paradigm that will elicit auditory recognition reliably. The task requires delayed matching of short segments of tape-recorded, trial unique sounds, such as noises, tunes, calls, sound-effects, etc. One of these sounds is played as the sample through an overhead speaker, and then after a short delay this one and another are played in alternation through two speakers that are widely separated on the test tray and that serve as the covers of the food wells. Two monkeys have now been successfully trained in the new paradigm, and one has already completed testing following damage to one of the two circuits of the limbic system, with little noticable effect. The evidence should thus soon be available as to whether the neural substrates of memory identified in the other modalities serve the auditory modality as well.

In addition to examining memory of stimulus quality in each sensory mode, we are also interested in studying memory of stimulus location, particularly the kind that might depend on the posterior parietal component of the dorsal visual processing pathway. Evidence from another project (MH-02035) has

indicated that this pathway, and the posterior parietal cortex in particular, is important for the perception of the spatial relations among objects. To determine whether the limbic system plays a role in memory for such relationships, and, by implication, whether the limbic system interacts with the dorsal pathways that mediate spatial perception as it does with the ventral pathways that mediate object perception, we are attempting to design a test for spatial memory. As in the case of auditory recognition, designing the test is proving extremely difficult, but given the potential theoretical value of such a test, continued effort is warranted.

(2) Associative memory

Earlier work had shown that performance on a task requiring the association of the visual and tactual qualities of an object was severly impaired by amygdalectomy but not by hippocampectomy. Conversely, performance on a task requiring the association of an object and its location was severely impaired by hippocampectomy but not by amygdalectomy. These findings suggest that one of the limbic system's major roles is in associative memory, with each limbic structure being responsible for the formation of associations between items processed in different pairs of sensory systems. Generalizing from our initial results, we may speculate that the amygdala mediates associations between items processed in the various stimulus-quality systems (i.e. stimulus-stimulus associations) whereas the hippocampus mediates associations between those items and items processed in the spatial-location systems (i.e. stimulus-place associations). New experiments to test this hypothesis are being planned, but many will require first the successful development of the nonvisual recognition tasks described above in (1).

Whether the foregoing principles apply to the association of two different items processed by the same sensory system is unclear. That question is posed by the results of experiments on one-trial object-reward association, a task that requires the animal to remember on the basis of a single presentation whether or not a trial-unique object had been baited with a piece of food. This task may be considered to be one of stimulus-stimulus association within vision. As might be expected from such an analysis, amygdalectomy was found to produce a more severe impairment than hippocampectomy. The impairment following amygdalectomy was recoverable however, and it became permanent only with the superimposition of hippocampectomy, indicating that the hippocampus also contributes to at least this form of stimulus-stimulus association. Further tests of associative memory within vision will be necessary to clarify the basis of this hippocampal contribution.

Using the same object-reward associative memory task, we have found that lesions of the inferior temporal cortex will not totally deafferent the limbic system of visual input unless tissue in the floor and possibly upper bank of the superior temporal sulcus is included in the ablation. This conclusion is based on the finding that although the standard inferior temporal lesion, which excludes the foregoing tissue, impairs object-reward association, the impairment, like that after amygdalectomy alone, is recoverable. The impairment becomes permanent, thereby resembling the impairment following combined amygdalo-hippocampal ablation, only when the inferior temporal lesion is extended to include the additional sulcal cortex. The implication of these

findings, beyond providing further support for the notion of cortico-limbic interaction in memory, is that disconnection by means of cortical lesions requires that the lesions be essentially total. Apparently, even relatively small segments of a cortical station can receive, process, and transmit sufficient information to carry on the functions of that station.

(3) Habit formation

At the same time that monkeys with limbic lesions are exhibiting rapid forgetting on both recognition and associative memory tests, they are nevertheless able to learn and retain multiple-trial object discriminations about as well as normal animals. This result can be obtained even when the monkeys are trained on 20 pairs of objects concurrently, and even though the successive trials on a given pair are separated by 24-hour intertrial intervals. This striking dissociation of effects following limbic lesions points to the existence of a powerful nonlimbic mechanism for learning and retention. We have postulated that this nonlimbic mechanism involves a cortico-striatal system and that the learning process for which it is responsible is habit formation.

In a separate project (MH-02039), we are attempting to examine the role of the neostriatum in habit formation by disrupting the entire nigro-striatal dopaminergic system with the selective toxin MPTP. In the present project, complementary experiments are underway in which selective electrolytic lesions are being made in just those portions of the neostriatum, i.e. posteroventral putamen and tail of caudate nucleus, that receive projections from the ventral visual processing system. In both cases, habit formation is being evaluated with the 24-hour concurrent object discrimination test described above.

Normal monkeys trained on this 24-hour concurrent discrimination test with numerous sets of objects learn the later sets much more rapidly than the earlier ones. To investigate the basis of this phenomenon, we are examining the effects of limbic lesions on the acquisition of numerous sets. If the monkeys with lesions also learn the later sets faster than the earlier ones, it would suggest that the increased learning speed simply reflects steady improvement in habit formation with continued practice. Should the animals with lesions fail to show the normal improvement, however, it would suggest that the normal monkey gradually learns to apply its cognitive memory ability over 24-hour intervals. In that case, the 24-hour concurrent discrimination test could become the basis of an extremely valuable new method for the study of long-term memory in normal monkeys, a form of memory that has thus far proven difficult to demonstrate.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

In the process of investigating the role of various temporal-lobe structures in the visual memory of monkeys, we obtained a result that is particularly exciting because if appears to solve the long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences or acquire new information, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this

syndrome in monkeys by removal of the hippocampus alone have largely failed. What we have found in our studies is that if damage to the hippocampus is combined with damage to the amygdala then a profound memory loss does ensue. The discovery has not only resolved the discrepancy between clinical and experimental findings in nonhuman primates, but has also provided new insight into the neural substrates of memory. Specifically, it has led to the development of a hierarchical model of recognition and associative memory involving a cortico-limbo-thalamic circuit that may well serve as the foundation for all cognitive processes beyond perception, including thought. As we gain further understanding of the memory system, and how it differs from the noncognitive system for habit formation, we will inevitably gain a better understanding of thought and its breakdown in normal and abnormal behavior.

PROPOSED COURSE OF RESEARCH:

Having found severe recognition losses in both object vision and touch after lesions of the limbo-thalamic system, we shall continue our attempts to devise tests of auditory recognition and visual spatial recognition, with the aim of determining whether the system is indeed critical for recognition in all perceptual modalities. Also, further attempts will be made to differentiate between amygdaloid and hippocampal contributions to associative memory, and we shall test whether any distinctions that are found are carried further through the thalamic and prefrontal segments of the two circuits. In addition, we shall continue our exploration of the neural basis of habit formation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02032-09 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neural coding of visual stimuli in the awake monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are recording from single neurons in the visual system of monkeys in the attempt to uncover the neuronal mechanisms underlying visual perception and visual recognition. One set of experiments carried out on neurons of inferior temporal cortex has shown that changing the attentional or cognitive demand of visual tasks changes the neuronal responses to a visual stimulus. For example, when the task was made more difficult so that the monkey committed more errors, the neuronal responses to the stimulus were increased. Also, neuronal responses to the stimulus differed depending on whether the animal was discriminating it from another on the basis of its texture or shape. In a second set of experiments, involving recording from single neurons of both inferior temporal and striate cortex, different visual stimuli were found to yield different temporal sequences of action potentials. We have developed quantitative techniques to identify which features of this temporal modulation are significant in carrying messages about stimuli. As a result, we have now been able to analyze the neuron as a communication channel carrying encoded information in the sequence of action potentials. The analysis showed that with a code that makes use of temporal modulation the neurons transmits twice as much information as when the code uses only the number of action potentials. The number of action potentials is not correlated with the information transmitted.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION:

Objectives:

Much of normal behavior is based on the recognition of what objects are and what they mean. We know from neurobehavioral studies that lesions of many cortical regions extending from the striate area to the inferior temporal area interfere with visual pattern discrimination and recognition. Presumably, single neurons in these brain regions carry on the requisite processing of the visual information. If we are to understand how single neurons transform and convey visual information we must have two types of knowledge. First, we must know what stimulus conditions influence the neuronal discharges in the relevant brain regions. And second, we must learn to recognize and decode the neuronal messages that carry information about those stimulus conditions. In our studies of these issues, we are recording from single neurons in the cortical visual system of awake, behaving monkeys.

In the first set of experiments we are studying the influences of different cognitive demands such as changes in attention or discrimination criteria on inferior temporal neuronal responses to visual stimuli. These experiments show that different cognitive requirements often lead to different strengths of discharge in response to a given stimulus.

In our second set of experiments we have continued the development of a method to assess the information conveyed by single neurons about individual visual stimuli. In the past we had noticed that a neuron's response to different stimuli often varied not only in the number of spikes but also in the pattern of spikes across time. This observation raised the possibility that temporal modulation of the spike train may convey information about stimulus features. The techniques we have been developing are designed to quantify the temporal modulation of neuronal responses, to identify the aspects of the spike trains that correlate with stimulus-related information (that is, to identify the neuronal code carrying information about stimulus conditions), and to quantify the amount and nature of the information transmitted.

Major findings:

For the first project, single neurons were recorded from inferior temporal cortex of rhesus monkeys while the monkeys performed three tightly controlled behavioral tasks. The different tasks required that the monkey's attention be directed differently while the visual stimulation and motor demands of these tasks remained constant. In all three tasks the monkey fixated a spot of light while one of four peripherally located visual patterns appeared on a screen. In the simplest task, the monkey was rewarded for making an immediate behavioral response to the dimming of the fixation spot, and it could thus completely ignore the peripherally located patterns. In the task of intermediate difficulty, the animal was rewarded for making an immediate behavioral response to the dimming of the peripherally located patterns, without having to distinguish between them, while continuing to gaze at the fixation spot. Finally, in the most difficult task, the monkey was rewarded for discriminating among the peripherally located patterns. These patterns

were either squares or circles with textures of either dots or stripes. If the stimulus shape was the relevant dimension, the monkey was rewarded for an immediate behavioral response to the onset of one shape, e.g. a circle, and with a delayed response to the onset of the other, in this case a square, regardless of the texture. Similarly, if the texture was the relevant dimension, the monkey was rewarded for an immediate behavioral response to the onset of one texture, e.g. dots, and with a delayed response to the other, in this case stripes, regardless of the shape. The monkey's eye position was closely monitored with the magnetic search coil technique to assure that the visual stimuli were presented at the same retinal location on each stimulus presentation. A given reinforcement contingency remained in effect for a block of trials before the reinforcement contingency was shifted for the next block of trials, and so on.

We obtained two important sets of results from these experiments. First, in 79 of 90 inferior temporal neurons recorded from two monkeys we found that the number of spikes that occurred in response to any single visual stimulus depended upon which task was performed. Further, we determined that the magnitude of the neuronal activity was directly related to the number of errors the monkeys made while performing the behavioral tasks. Both the number of spikes and the number of errors were lowest during the fixation task, intermediate during the peripheral attention task, and greatest during the pattern discrimination task. Thus, there exists a large group of inferior temporal neurons whose responsiveness is modulated in proportion to the number of errors made in different tasks. The level of responsiveness could thus be related to either task difficulty or to the monkey's level of attentiveness, since it is possible that the monkey is more vigilant in more difficult tasks.

The second finding from these experiments was that changing the dimension of the stimulus that was relevant for discrimination changed the neuronal response to that stimulus. For example, the neuronal response differed depending on whether the striped circle was discriminated by its shape or by its texture. This effect occurred in 25/52 neurons studied. Further, for each neuron, we determined which of the four compound stimuli yielded the largest difference between texture and shape discrimination trials, and noted which feature of the stimulus yielded the larger response. We then checked whether the other compound stimulus containing the same feature also elicited a larger response when the dimension to which that feature belonged was the relevant one. A consistent response pattern of this kind was seen in 91% of the neurons studied. These results show that different attentional demands influence the responses of inferior temporal neurons to visual stimulation.

In addition to changes in response magnitude, many neurons showed temporal modulation in response to visual stimuli. As a result, single inferior temporal neurons often could not be categorized as phasic or tonic, nor could a single latency be assigned. Furthermore, the different temporal patterns suggested that the temporal modulation of the spike train as well as the number of spikes might contain information about the stimulus. To investigate the significance of temporal modulation we developed an unbiased method of quantification which made no assumptions about which features of the spike trains might be carrying messages about the stimuli. The method identified

those features of the spike trains that might contain messages and how many independent features would be needed to describe the messages.

The data for the analysis were obtained from inferior temporal neurons in rhesus monkeys trained to fixate a spot in the center of a tangent screen. Each one of a large set of two-dimensional black and white stimuli were presented for 400 ms. while the fixation point was momentarily blinked off. The set consisted of 128 stimuli derived from a complete set of orthogonal, two-dimensional Walsh functions. Through appropriate weightings of its members, an orthogonal basis set can be used to reconstruct any black and white image. The use of this set means that every possible two-dimensional visual feature is represented in the stimulus set. Therefore, in principle, a complete representation of a neuron's responsivity to two-dimensional features can be obtained.

The individual spike trains were first converted into continuous spike density functions which estimate the probability of spike occurrence at any point in time. The spike density functions were then quantified by means of principal component decomposition. The principal components, which are an ordered set of orthogonal transform elements derived from the data themselves, are specified so that, as each successive one is added to the description of the data, the residual error is the least possible for that number of data descriptors. Therefore, the ordering of the principal components shows which features are the most important. Also, the principal components yield sets of coefficients that are uncorrelated with each other, and consequently the coefficients can be tested with univariate statistical methods to determine whether any of the temporal patterns represented are correlated with the stimuli or whether they represent random fluctuations in the spike trains.

The principal component analysis unexpectedly showed that inferior temporal neurons seem to fall into only two groups based on the forms of their first principal components, which are either phasic or tonic. The derivation of the principal components requires that they be orthogonal to each other, but they are otherwise unconstrained in form and depend only upon the data set. Hence, they could, in principle, assume any form. Nonetheless, the 21 inferior temporal neurons we have tested thus far with this method had first principal components that belonged to only one of these two sets, ten in one group, eleven in the other. The first principal component, being a weighted average of the response, was highly correlated with the spike count for all neurons, whereas the later principal components were very poorly correlated with spike count.

The complete set of principal components gives a complete multivariate description of the neuronal responses. By adapting a computational statistical method called the bootstrap method, we have been able to show that each of the first three to four principal components has a systematic association with the stimulus displayed. This indicates that temporal modulation was partly independent of response magnitude, since only the first principal component correlates with response magnitude. Thus, the stimulus dependent features of the spike trains are not completely represented by a univariate measure, but only by multiple parameters, probably 3 to 4. One interpretation of these results is that a neuron can be regarded as a filter

that maps visual stimuli from a picture space with many dimensions to a response space with many fewer dimensions, though not a single one.

On the basis of these results we hypothesized that temporal modulation conveys significantly more information about stimuli than does response magnitude alone. In order to test this hypothesis, we developed methods to allow the application of Shannon's information theory. Information theory is a statistical/mathematical formalism that quantifies the input-output relationships of a communications channel. To apply the formalism of information theory, the neuron is regarded as a communication channel that associates an input code, in this case the visual patterns, with an output code, in this case parameters of the spike train, through a statistical or probabilistic relationship. It assumes nothing about the nature of the association nor does it assume that the communication system is linear. Previous applications of information theory to neuronal responses have in general assumed binary codes and have dealt mainly with channel capacity and temporal distribution of information. These approaches did not examine the nature of the neuronal code elements. In our application of information theory, we have used the Walsh patterns to form an input code and the principal components to form a multivariate output code representing information about the stimuli. Our hypothesis, then, is that an orthogonal set of temporal response waveforms represented by the principal components is used by single neurons to transmit information about stimulus features. In order to apply information theory, we developed a method for deriving the requisite discrete probability matrix from the continuous distribution of principal component coefficients. The average transmitted information and the information per stimulus could then be calculated from the joint probability density function of stimulus-response pairs.

Both the spike count alone (a count code) and the first three principal components (a three-dimensional temporal code) were used to form two different output codes. Average information transmitted by the count code was 0.43 +- 0.04 bits (SE, N = 21), whereas by the temporal code it was 0.88 +- 0.05 bits. Thus, the three-dimensional code transmits twice as much information as the unidimensional count code.

Although the spike count alone transmits half the information contained in the spike train, the spike count itself is not correlated with the information transmitted (r = 0.18, +- 0.04). This is an important finding since it indicates that the spike count itself cannot be regarded as a direct estimate of the information transmitted by a single neuron. Rather, the probabilistic input/output relationship must be calculated to quantify the information transfer. Since the principal components fell into two groups, we infer that there may be a universal code for representing stimulus features. This suggests that the spike train contains a multiplexed message about the stimulus that is present.

We have carried out similar experiments in striate cortex. The results have not yet been as extensively analyzed as the ones from inferior temporal cortex, but it is clear at this time that the results are qualitatively similar. That is, there is stimulus dependent temporal modulation. Again, three to four parameters characterize the temporal modulation, and when the

information is assessed the neurons transmit about 0.4-0.5 bits of information in a count code and about 0.85-1.0 bits in a three-part temporal code. The analysis of these data is continuing.

The results of our analysis are inconsistent with one of the traditional views regarding the neural code, namely, that individual neurons are feature detectors with firing rates indicating the confidence that a particular trigger feature is present in the receptive field. The results are also at odds with the view that unidimensional tuning curves describe the information transmitted by a single neuron since multiple features of the spike train are modulated independently by different stimulation conditions. The multidimensional nature of the stimulus-response relationship suggests that there are no optimal features; instead, the neuron is continuously transmitting an encoded multidimensional representation of the stimuli. The results lead to an hypothesis which might be relevant throughout the entire visual system. Neurons appear to encode multidimensional spatial properties by means of multivariate patterns of temporal modulation as information containing codes. Interpretation of the behavior of an ensemble of such neurons may depend not only upon the magnitude of the responses in individual units but also upon the coherence of the temporal sequences converging from the ensemble onto later neurons. It appears in our first mathematical explorations of this ensemble model that decoding a multiplexed message may be computationally easier because of smaller possible ambiguity than decoding unidimensional messages.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Many psychiatric and neurological disorders are accompanied by disordered attention, perception, and memory. The goal of this project is to gain an understanding of the mechanisms that normally underlie these basic cognitive processes. That understanding should aid in developing strategies for effective palliative treatment of cognitive deficits and for restitution of cognitive function.

PROPOSED COURSE OF RESEARCH:

The investigations of the attentional influences on visual responses of inferior temporal neurons will continue. The experiments will require several more months of work, after which the data will be analyzed and prepared for publication.

The investigations on information transmission in the visual system has led to many new concepts about how individual neurons function. Some of these ideas must be reconciled with the known properties of visual system neurons. For that purpose new experiments are being readied which will allow direct comparison between our model and others. These experiments will first be undertaken in striate cortex because of the large body of information available about the properties of striate neurons.

PUBLICATIONS:

Richmond, B.J. and Goldberg, M.E. On computer science, and the physiological utility of models. Behav. Brain Sci. 8: 300-301, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02033-08 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional mapping of sensory systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Laboratory of Psychology and Psychopathology, NIMH

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Laboratory of Neuropsychology

SECTION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cerebral areas related to vision in the rhesus monkey were identified and delineated by applying the [^{14}C] 2-deoxyglucose method and comparing metabolic activity (local rates of glucose utilization) in visually stimulated versus visually deafferented cerebral hemispheres. The visual-nonvisual borders of 1) an occipito-temporal visual pathway, and 2) an occipito-parietal visual pathway were determined and, further, their points of interaction with inferior prefrontal cortex, prearcuate cortex, and with limbic, striatal, and diencephalic structures were specified. The functional contribution of the forebrain commissures to vision was quantified by comparing monkeys prepared with complete vs. partial visual deafferentation of one hemisphere (i.e. optic tract section plus forebrain commissurotomy vs. optic tract section alone, respectively). Some of the cerebral areas serving multimodal functions were identified through a comparison of brain metabolism in monkeys given visual stimulation only and monkeys given visual plus somatosensory stimulation. Finally, the functional development of the visual system was traced in a series of infant monkeys. In these animals, metabolic activity appears to reach adult levels at about 4 months of age.

PROJECT DESCRIPTION:

Beyond primary visual cortex, two main cortical pathways are known to be critical for higher-order visual functions. One is a ventral pathway which proceeds from striate through prestriate to inferior temporal cortex, and from there to inferior prefrontal cortex, and is specialized for object vision. The other is a dorsal pathway which proceeds from striate through prestriate to inferior parietal cortex, and from there to the prearcuate region of dorsal prefrontal cortex, and is specialized for spatial vision. Both pathways project to various thalamic, limbic, and striatal structures. Previously unknown details regarding the full extent and exact boundaries of this cortical tissue, as well as points of contact with subcortical forebrain targets, were revealed in a comprehensive picture of the entire visual system at work, achieved by applying the [^{14}C] 2-deoxyglucose method.

Experiment 1:

To map the visual system at work, we prepared monkeys with a tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. The comparison of local cerebral glucose utilization (LCGU) in a "seeing" vs. a "blind" hemisphere within the same animal made it possible to identify and delineate the areas related to vision.

The 2-deoxyglucose method was applied while monkeys, restrained in a primate chair, either 1) passively viewed a high-contrast geometric pattern mounted on a rotating drum that surrounded them, or 2) actively performed a visual pattern discrimination task that required a response with the hand opposite the blind hemisphere, correct responses being reinforced with a water reward.

Both conditions of visual stimulation revealed reduced metabolic activity in the "blind" right as compared with the "seeing" left hemisphere not only in the geniculostriate system but throughout all of the ventral cortical visual pathway, namely, prestriate, inferior temporal, and inferior prefrontal cortex. The areas of depressed LCGU included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe, side-to-side differences were seen in lateral and basolateral amygdala, posteroventral putamen, ventral claustrum, and tail of caudate. Subcortically in the temporal lobe, hemispheric differences were seen in the anterior part of the head of the caudate nucleus. Asymmetries were also seen in the dorsal cortical visual pathway, namely, the inferior parietal lobule and the prearcuate region of the frontal lobes. Subcortically, both the body and the posterior portion of the head of the caudate nucleus, known to receive input from posterior parietal and prearcuate frontal cortex, showed right hemispheric reductions in LCGU. Performance on the discrimination task led to an asymmetrical increase in LCGU in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking. In visual areas, each condition of visual stimulation produced essentially the same result. In certain subcortical visual areas, however, the monkeys performing the visual discrimination task showed a lack of left-right hemispheric asymmetries compared to those of the passively

stimulated group. Thus, in the body and head of the caudate nucleus, the lateral and basal amygdala, and the medial pulvinar, left-right asymmetries virtually disappeared in the actively discriminating animals, due mainly to increases in right hemispheric activity presumably related to asymmetrical somatosensory input from the active hand. Since visual activation of these regions in the left hemisphere was balanced by somatosensory activation of the same regions in the right hemisphere, it appears that these are zones of sensory convergence serving multimodal functions.

The limits of cortical visual tissue were marked by sharp changes in LCGU, and were highly consistent among the animals. Computer-enhanced images of the autoradiographic brain sections were examined, and the exact visual-nonvisual borders of visually related tissue in the parietal and temporal lobes were delineated, revealing more visual tissue than expected in both regions.

Behind the junction of the lunate and the intraparietal sulcus all cortical tissue is related to vision. In front of this junction, nonvisual tissue, probably related to somatic sensation, first appears in the superior parietal lobule. Where the lateral fissure begins, auditory tissue appears, separating the visual tissue into the occipitoparietal and occipitotemporal pathways.

In the parietal lobe, the upper border is always within the intraparietal sulcus, about halfway down the upper bank caudally and closer to the fundus rostrally. The lower border moves out of the lateral fissure, and then it moves into the intraparietal sulcus rostrally. The rostral limit of visual tissue is within the intraparietal sulcus, about 5mm behind its anterior tip.

In the temporal lobe, the upper border is always within the superior temporal sulcus, generally about halfway down the dorsal bank caudally but within the fundus rostrally. The lower border moves from the calcarine fissure to the hippocampal sulcus (where it continues midway along its length) and then turns laterally to enter the occipitotemporal sulcus and finally the fundus of the rhinal sulcus.

These visual-nonvisual borders generally appear at zones of cytoarchitectonic transition described by Bonin and Bailey. For example, in the parietal lobe, a visual-nonvisual border appears on the lateral surface near the zone of transition between areas PG and PF and on the medial surface between prestriate area OA and parietal area PE. Also, in the temporal lobe, visual-nonvisual borders appear in the transition zones between TF and TH, TE and TH, and TE and TG. Finally, inside the expanse of visually related cortex, metabolic borders appeared to separate archictecturally different subareas, as in the lower bank of the intraparietal sulcus and in the upper bank of the superior temporal sulcus. These results lend new functional validity to cortical architectonics.

Experiment 2:

The forebrain commissures reciprocally connect parts of each visual area within the ventral cortical visual pathway. Specifically, the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of areas TEO and TE receive

contralateral input via both the splenium and the anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we attempted to localize and to quantify the contribution to vision made by the commissural systems. To do this we prepared monkeys with a unilateral optic tract section alone leaving the forebrain commissures intact. The 2-deoxyglucose method was applied one month postoperatively under the two behavioral conditions described in Experiment 1. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of these monkeys and those from Experiment 1 with the forebrain commissures sectioned.

An extensive computerized quantification of the ventral cortical visual pathway of the animals from Experiments 1 and 2 revealed that in the intact hemisphere all animals showed a sequential decline in LCGU along the cortical visual pathway from a high of 66 umoles/100g/min in area OC to a low of 47 in anterior TE. Moreover, there were no statistically significant differences in these measures resulting either from the differences in conditions of visual stimulation or in surgical preparation. In the deprived hemisphere there were again no significant differences due to the differing behavioral conditions. There was, however, a statistically significant interaction between surgical preparation and cortical area. In areas OC through TEO, LCGU averaged 50% of that in the intact hemisphere for all animals. In area TE, however, LCGU remained at 60% of that in the intact hemisphere in animals with sectioned commissures but increased to an average of 85% in animals with the commissures intact. This increase in LCGU clearly reflects the functional contribution of the forebrain commissures to vision, but, surprisingly, only to area TE and not to the posterior zones of the pathway, areas TEO, OA, and the OC-OB border. In this prestriate-posterior temporal zone, it was possible that commissural input would be effective only against a background of direct excitation from the retina. To test this possibility, we prepared a control group of monkeys in which a "blind" right hemisphere was produced by midline section of the optic chiasm combined with occlusion of the right eye, rather than by section of the optic tract. This preparation, however, also failed to reveal increased metabolic levels in the prestriate-posterior temporal zone of the visually occluded hemisphere. Clearly, the presence of background spontaneous neural activity provided by an intact retinal projection did not augment or change the commissural contribution to vision in areas TEO, OA, or the OC-OB border. The likely explanation for the failure of commissural fibers to activate glucose metabolism in the posterior portions of the occipito-temporal pathway has been suggested by evidence gathered in another project from this laboratory (MH 02036). That evidence strongly suggests that the primary function of the commissures in the posterior portion of the visual system is to provide suppressive rather than excitatory influences on neural activity.

Experiment 3:

The 2-deoxyglucose method was used to trace the functional development of the visual system. A series of infant monkeys were prepared with unilateral optic-tract section combined with forebrain commissurotomy at 1 day, 1 week, and 1, 2, 3, and 5 months of age. The 2-deoxyglucose method was then applied during conditions of passive visual stimulation as described in the first

experiment. The reults show that there are systematic age-related changes both in the absolute level of LCGU within the normal seeing hemisphere and in LCGU differences between the normal left and the deprived right hemispheres.

In all cortical visual areas of the intact hemisphere, LCGU was lowest in the youngest subjects, peaked at 4 months, and then declined in the 6-month-old subject to levels found in adults. As in adults, the intact hemisphere of infants shows a progressive decline in LCGU along the ventral cortical visual pathway from a high in area OC (ranging from 26.1 umoles/100g/min at 9 days to 88.1 at 4 months) to a low in anterior TE (ranging from 17.6 at 2 days to 59.7 at 4 months). This gradient was present in all subjects, but was shallowest in the two youngest.

The deprived hemisphere showed reduced LCGU relative to the normal hemisphere in all areas of the cortical visual pathway at all ages. Also at all ages, hemispheric differences were greatest in area OC and smallest in anterior TE. These differences, however, varied systematically with the age of the animal. Thus, for each cortical area, the relative difference between the normal and deprived hemispheres was smallest in the youngest subjects and approached the differences seen in adults only at about four months of age, the time at which LCGU appeared to peak.

This finding that adult levels of metabolic activity are not reached until about 4 months of age is consistent with behavioral data (see Project MH 02038) indicating that adult levels of visual object recognition probably do not develop until about this time. Additional animals of various ages are now being prepared and studied in order to achieve a more detailed analysis of this developmental sequence.

Experiment 4:

Because the two conditions of visual stimulation outlined in Experiment 1 yielded virtually identical results, we are attempting to tax the visual system further by including additional conditions of visual stimulation.

Two monkeys are being trained to perform a running recognition task that requires the continual storage and retrieval of the memory of objects, thus taxing visual memory throughout the experimental session. While restrained in a primate chair, these animals are rewarded with water for correct discrimination of novel vs. familiar stimuli within each experimental session.

Once a performance criterion of 90% correct is achieved, these animals will be prepared with combined optic tract section and forebrain commissurotomy as before. Finally, the 2-deoxyglucose method will be applied in order to measure metabolic activity throughout the visual system while visual memory is being taxed. We expect to see increased LCGU in parts of the limbo-thalamic system that did not exhibit this increase in our purely visual perceptual studies.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The goal of this research is a better understanding of normal function within

the central nervous system, a goal that will in time aid in diagnosing and treating the abnormal function underlying a variety of mental disorders. Since it has been widely demonstrated that metabolic activity, in the form of glucose utilization, and functional activity are highly correlated within the central nervous system, the 2-deoxyglucose method provides a unique method of relating neural structure and function. This method permits for the first time both the visualization and quantification of local levels of metabolic activity simultaneously throughout the entire brain in animals studied either under normal conditions or following experimental intervention. The results continue to provide important insights into the role of various cerebral structures, both cortical and subcortical, in particular behaviors. Our initial studies have contributed information regarding both primary and higher-order visual processing, the understanding of which is critical for the diagnosis and treatment of sensory, perceptual, and mnemonic disorders related to vision.

PROPOSED COURSE OF RESEARCH:

Our immediate goals are to continue the investigation of the extended visual system in primates including 1) delineation of the visual-nonvisual borders in the frontal lobes, 2) a more extensive quantification of the dorsal visual pathway, and 3) investigation of the visual system while visual memory is being taxed.

We also plan to continue our investigation of the development of the visual system in infant monkeys, first completing the normative study under conditions of passive visual stimulation and then attempting to parcel out developmental differences between the "habit" and the "memory" systems (see Project MH 00478). Ultimately, our goal is to apply the 2-deoxyglucose method to the study of a variety of behavioral processes in the adult and infant monkey, including perception, attention, memory, emotion, and volition, for the purpose of identifying the various structures involved in these different behaviors and quantifying the degree of their participation.

(The principal investigator of this on-going research project assumed part-time guest researcher status in May 1983; consequently, progress has been slowed due to limited man-hours.)

PUBLICATIONS:

Macko, K.A. and Mishkin, M. Metabolic mapping of higher-order visual areas in the monkey. In L. Sokoloff (ed.): Brain Imaging and Brain Function, Raven Press, New York, pp. 73-86, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02035-05 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Anatomy of the primate visual system

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

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LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To better understand the role of visual association cortex in perception and memory, we have been examining the multiple functional areas that comprise this cortex in the macaque and exploring the complex circuitry of their interconnections. In these studies, we have utilized neuroanatomical tracing techniques combined with electrophysiological recording as well as newly developed histological stains that, for the first time, clearly distinguish among the multiple visual areas. Our results indicate that the primary visual area, striate cortex, is the source of two cortical projection systems. The first system begins with the striate projections to the second and third visual areas, V2 and V3. Both V2 and V3 project in turn to V4. These three prestriate areas are arranged in adjacent belts that nearly surround the striate cortex, and, like the striate cortex, each belt contains a topographic map of the visual field. Area V4 projects in turn to the inferior temporal cortex. The second system begins with both striate and V2 projections to area MT, which is also topographically organized. However, in contrast to V4, which appears to provide a major link forward from striate cortex into the temporal lobe, our results on MT indicate that it provides a major link forward from striate cortex into the parietal lobe via its projections to four additional areas in the superior temporal and intraparietal sulci. Thus, one system of projections out of striate cortex is directed ventrally into the temporal lobe, while a second is directed dorsally into the parietal lobe. On the basis of evidence from our neurobehavioral work, we propose that these two systems mediate object vision and spatial vision, respectively. We have begun an analysis of human brain material with the goal of identifying visual areas homologous to the ones that we have delineated in the macaque.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION:

To understand the role of visual association cortex in perception and memory, we must identify the multiple functional areas that comprise this cortex, delineate their topographic organization, and explore the complex circuitry of their interconnections. So far, we have discovered that the primary visual area, striate cortex, is the source of two divergent corticocortical pathways, each with its own set of hierarchically organized prestriate association areas. One pathway is directed ventrally into the temporal lobe, whereas the other is directed dorsally into the parietal lobe. On the basis of evidence from our neurobehavioral work, we propose that these two pathways mediate object recognition and spatial perception, respectively. Our most recent studies suggest that both the temporal and parietal lobes may each consist of multiple visual areas, and we are currently investigating this possibility. Finally, we have begun an analysis of human brain material with the goal of identifying visual areas homologous to the ones that we have delineated in the monkey.

An occipitotemporal pathway for object recognition

To trace the flow of visual information through the cortex, we have employed both anterograde and retrograde tracing techniques, such as autoradiography and horseradish peroxidase histochemistry, in combination with electrophysiological recording. To establish the direction of flow of information and thereby the hierarchical arrangement of cortical visual areas, we have examined the laminar pattern of connections between areas. Thus, "forward" projections, originating from cells located in the supragranular layers and terminating in granular layer 4, characterize not only the direct projections of striate cortex itself but also the cortical projections of visual association areas similarly directed away from striate cortex. Likewise, "backward" projections, originating from cells in the infragranular layers and terminating in layers excluding granular layer 4, characterize the cortical projections of visual association areas directed towards the striate cortex.

Our results indicate that the major outputs of striate cortex, or V1, are to areas V2, V3, and MT. Since V2 and V3 are associated with both the occipitotemporal and occipitoparietal pathways, whereas MT is associated with the occipitoparietal pathway only, the projections to V2 and V3 will be described first. The largest projection field of V1 is V2, a visuotopically organized area that nearly surrounds V1. There is, in addition, a much smaller and weaker projection from the lower visual field representation of V1 to the lower visual field representation of V3, a narrow area bordering V2. The vertical and horizontal meridians are represented alternately at the anterior borders of V1, V2, and V3, respectively.

The major projections of areas V2 and V3 are to V4 and MT. The visuotopic organization of V4 roughly parallels that of V2 and V3, in that the central visual field is represented laterally in the hemisphere, the peripheral visual field is represented medially, the upper visual field is represented ventrally, and the lower visual field is represented dorsally. The projections of V2 and V3 to MT are in topographic register with the one MT receives directly from V1.

Although V4 has minor projections to both MT and a small zone in the parietal cortex, its major output is to the inferior temporal (IT) cortex. Physiological studies have shown that, unlike the visual areas that precede it, IT cortex has no discernible visuotopic organization. Rather, IT neurons have very large receptive fields that nearly always include the center of gaze and frequently cross the vertical meridian into the ipsilateral visual field. Thus, IT cortex appears to be the "last" visual area in the cortical system for object recognition, as its cortical outputs are to areas in the temporal and frontal lobes that are probably not exclusively concerned with vision. Interestingly, IT cortex appears to receive a very complex pattern of projections from V4, with a single site in V4 projecting to several separate fields which interdigitate with projection fields of other sites in V4. Thus, there may be a mosaic of visual areas within the temporal lobe, and we are currently investigating this possibility. As described below, our results on the projections of MT suggest that there may also be a mosaic of visual areas within the parietal lobe.

In summary, the occipitotemporal pathway consists of areas V1, V2, V3, V4, and IT cortex. Neurobehavioral studies have shown that lesions along this pathway produce severe impairment in a wide variety of pattern and object discrimination tasks, and electrophysiological studies have shown that neurons in areas along this pathway are tuned to the qualities of a visual stimulus, such as shape and color.

An occipitoparietal pathway for spatial perception

Although area MT receives inputs from areas that participate in the occipitotemporal pathway (i.e. V1, V2, V3, and V4), its outputs appear to be mainly to areas located in the intraparietal and superior temporal sulci that project in turn to the inferior parietal lobule. Thus, MT appears to provide a major link from V1 into the parietal lobe. Within the intraparietal sulcus, a posterior projection zone (V3A) begins in the annectent gyrus caudally and entends along the fundus of the posterior third of the intraparietal sulcus. We had found earlier that this zone also receives a sparse projection from V1. The other projection zone in the intaparietal sulcus (VIP) lies in the anterior two-thirds of the sulcus, extending fron the fundus onto the posterior bank. Within the superior temporal sulcus, one projection zone of MT (MST) is located on the anterior bank of the sulcus, bordering MT medially, and the other (FST) is located in the sulcal floor, bordering MT anteriorly. There is a great deal of overlap in the projections to these medial and anterior zones from all parts of MT, indicating a convergence of inputs representing widely separated parts of the visual field.

The finding of multiple projection zones of MT in both the superior temporal and intraparietal sulci raises the question of what role these areas play in visual function. To explore this question, we have begun recording the electrophysiological properties of neurons within MT's projection zones and comparing these properties with those of neurons in MT itself. Our results thus far indicate that area MT, defined as the heavily myelinated portion of the V1 projection zone in the superior temporal sulcus (STS), contains a systematic representation of only about the central 30°-40° of the contralateral visual field. The far peripheral field is represented medial to

MT in MTp, which we had earlier found receives projections from the far peripheral field representations in V1 and V2. Like MT, MTp contains a high proportion of directionally selective cells, and receptive field size in MTp is the size expected of MT fields if the latter were to extend into the periphery. Areas MST and PP are found medial to MT and MTp. Both MST and PP have a high proportion of directionally selective cells, but only MST receives a direct projection from MT. Cells in MST have larger receptive fields than those in either MT or MTp but nonetheless display a crude visuotopic organization. Receptive fields of cells in PP are even larger, some including the entire contralateral visual field. Furthermore, unlike cells in MST, some in PP repond to auditory or somesthetic stimuli in addition to visual. Area FST, anterior to MT in the fundus of STS, also receives a direct projection from MT, but only about a third of its cells are directionally selective. Receptive fields of cells in FST are large, often include the center of gaze, and often cross into the ipsilateral visual field.

The neuronal properties of areas in the caudal STS suggest that MT, MTp, MST, and PP, together with the superior temporal polysensory area, constitute a cortical system for motion analysis. At successive stages of this system, neurons appear to integrate motion information over progressively larger portions of the visual field, respond selectively to more complex types of motion, and respond to inputs from additional sensory modalities. In addition, these areas may become increasingly involved in visuomotor control, inasmuch as lesions along the occipitoparietal pathway cause impairment not only in spatial perception but also in eye movements and visually guided hand movements as well.

Comparison of human and nonhuman primates

Our studies in the rhesus monkey indicate that: 1) visual cortex extends far beyond the striate cortex into both the temporal and parietal lobes; 2) within this cortex there exist multiple, functionally distinct areas, each with its own representation of the visual field; and 3) these areas are organized into two separate pathways, one for the recognition of objects and the other for the appreciation of the spatial relationships among objects. Despite the fact that these findings have been demonstrated in all monkey species studied thus far, suggesting a common primate plan, our concepts of human visual cortex have not progressed since the turn of the century. Thus, current neurological literature still refers to human visual cortex as containing a primary area (area 17 or striate cortex) and only two secondary prestriate areas (18 and 19). In an attempt to extend our concepts from monkey to human visual cortex, we have begun to examine human brain material.

In our first study, we investigated the inferior longitudinal fasciculus (ILF), commonly considered to be a long association fiber bundle interconnecting the occipital and temporal lobes. Based on blunt dissections of human and monkey brains, we found that the only long fiber bundle common to both the occipital and temporal lobes is the geniculostriate pathway (i.e. optic radiations) located within the external sagittal stratum. In addition, our autoradiographic experiments in monkeys indicated that the pathway from occipital to temporal cortex consists of a series of U fibers which course beneath the cortical mantle to connect adjacent regions in striate,

prestriate, and inferior temporal cortex. By implication, the occipital and temporal lobes in human beings are similarly connected by a series of U fibers, and we therefore suggest that the term ILF be replaced with the term occipitotemporal projection system. Different clinical syndromes that are attributed to ILF lesions, including visual agnosia, prosopagnosia, and loss of recent memory in vision, are probably due to interruption of fibers at different points along this projection system.

In our second study, we attempted to identify the multiple visual areas in the human cortex by applying the same histological stains that have been developed to differentiate among the multiple visual areas in the monkey cortex. Despite our best efforts, however, it has become clear that it will be necessary to study an intermediate species among the great apes in order to bridge the gap between monkey and man.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

An understanding of the basic mechanisms mediating normal visual perception and memory is the first step in the prevention, diagnosis, and alleviation of sensory, perceptual, and mnemonic disorders. To this end, we have been exploring projections out of the striate cortex to prestriate association areas. Our goal has been to trace the complex system of projections stepwise to the still higher-order visual areas located within the temporal and parietal lobes, areas critical for object vision and spatial vision, respectively. The combined use of axonal transport techniques and electrophysiological recording provides a powerful tool for tracing neural connections within these central visual pathways. In addition, the recent development of highly selective histological stains may give us the opportunity for the first time of identifying higher-order visual areas in the human brain that we have identified in the monkey.

PROPOSED COURSE OF RESEARCH:

Thus far, we have found that visual cortex in the monkey is organized into two divergent corticocortical pathways and that the projections of both pathways can be traced from the striate cortex through multiple prestriate association areas to the still higher-order visual areas in the temporal and parietal lobes. Our recent studies suggest that both the temporal and parietal lobes may also consist of multiple visual areas, and we will continue to investigate this possibility. Since there are no direct connections between temporal and parietal cortex, a major question to be answered is how the object and spatial information carried in these two separate pathways are subsequently integrated anatomically to yield a unified percept. Finally, we will explore the links of both pathways to affective, memory, and motor systems by examining the projections of the multiple visual association areas to the limbic system, the prefrontal cortex, and the striatum.

PUBLICATIONS:

Tusa, R.J., and Ungerleider, L.G. The inferior longitudinal fasciculus: A re-examination in man and monkey. Annals Neurol. (in press), 1985.

Ungerleider, L.G. The corticocortical pathways for object recognition and spatial perception. In C. Chagas, R. Gattass, and C. Gross (Eds.) Pattern Recognition Mechanisms, The Pontifical Academy of Sciences, Vatican City, pp. 21-37, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02036-05 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural representation of visual stimuli in the extrastriate cortex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The neural mechanisms for the visual recognition of objects extend beyond striate cortex into multiple extrastriate cortical areas within the occipital, temporal, and parietal lobes. To understand the neural mechanisms of perception and memory in these areas, we are studying 1) passive sensory coding by single neurons in the immobilized monkey 2) dynamic aspects of coding by neurons in an awake monkey engaged in a task requiring selective attention and memory and 3) the functional architecture of the cortex utilizing local metabolic mapping techniques. In the anatomical pathway that mediates spatial perception, we have found that neurons in one area, MT, are arranged in direction-of-motion columns, similar to the orientation columns discovered in the primary visual cortex. In the anatomical pathway that mediates object recognition and memory, we have found that neurons in area V4 code many local features of objects, such as the length and width of contours, textures, and colors. Since neurons in this area are sensitive to form and color differences between a stimulus and its background, they may play a role in separating figure from ground. One of the primary functions of the corpus callosum in area V4 appears to be to integrate the figure/ground mechanism across both halves of the visual field. Neurons at the next stage of processing, inferior temporal cortex, are sensitive to the global properties of objects, such as their shape. In both area V4 and the inferior temporal cortex, we have found that selective attention gates visual processing by filtering unwanted information from the receptive fields. By contrast, selective attention has no effect on neurons in the primary visual cortex or in V2. Based on these results, we propose a two-stage model of perception. At the first stage, figures are extracted from their background by a mechanism that works in parallel over the retina and is preattentive. At the second stage, the relevant figure is selected to be remembered or acted upon by a mechanism that works serially over the retina and is attentive.

PROJECT DESCRIPTION:

Previous work in this and other laboratories has shown that the cortical systems involved in perception and memory include multiple visual areas that lie beyond the striate cortex within the occipital, parietal, and temporal lobes. To begin to understand the neural mechanisms of perception and memory in these extrastriate areas, we have taken three interrelated approaches. The first is to establish the basic sensory information coded by neurons in the multiple extrastriate areas of macaques. The second is to study the activity of neurons in these extrastriate areas and in subcortical structures while the monkey is engaged in tasks requiring selective attention and memory. Finally, the most recent approach has been to explore the functional architecture of the visual association cortex utilizing local metabolic mapping techniques.

Sensory Coding

Extraction of figure from ground in area V4

Anatomical experiments in our laboratory have shown that visual area V4 is a central station in the pathway from the primary visual cortex to the object recognition system of the temporal cortex. To study the passive visual properties of neurons in V4, unaffected by eye movements or the changing state of the animal, we initially recorded V4 neuronal responses in the immobilized, lightly anesthetized macaque. In experiments which are described later in this report, we have begun to examine the dynamic aspects of neuronal coding in V4 in the awake, behaving animal. Although V4 was originally thought to be exclusively concerned with color, we found that V4 neurons are as sensitive to stimulus form as they are to color. In fact, V4 neurons are at least as sensitive as neurons in the primary visual cortex to such features of form as the orientation and size of contours and the spatial frequency of sine wave gratings. Ours are the first findings to indicate that V4 is far more than a "color processing area".

What are the special contributions of V4 to perception? We have found that unlike neurons in the primary visual cortex, the receptive fields of neurons in V4 are surrounded by large, silent suppressive regions with specific form and color properties. Stimuli placed outside of the receptive field do not elicit any response from a V4 neuron, yet these stimuli are able to completely suppress the response of the neuron to a similar receptive-field stimulus. Thus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or form. These neurons may thus play a role in separating 'figure' from 'ground', a fundamental task in visual perception.

The discovery of large suppressive zones surrounding the receptive fields of V4 cells may explain the function of heavy commissural projections to V4 found in our anatomical experiments. Like other prestriate areas, V4 contains a representation of only the contralateral visual field. Within the central visual field, V4 receptive fields rarely extend more than 1° across the vertical meridian into the ipsilateral visual field. Yet, V4 receives heavy commissural projections from the opposite hemisphere not limited to the representation of the vertical meridian. To test whether the commissural

projections were related to the suppressive surrounds in V4, we measured the extent of the suppressive surrounds of individual V4 neurons within the ipsilateral hemifield. We found that even though the excitatory receptive fields of V4 neurons were confined to the contralateral hemifield, the suppressive surrounds extended up to 16° across the vertical meridian into the ipsilateral visual field. The ipsilateral suppression was eliminated following section of the corpus callosum. Thus, the commissural inputs to V4 (and presumably other prestriate visual areas) appear to be largely suppressive and may serve to integrate the figure/ground mechanisms in the two hemispheres. Recently, we have found that the suppressive surrounds of cells in striate (primary visual) cortex are much smaller than those in V4 and do not extend into the ipsilateral visual field, suggesting that the separation of figure from ground is primarily an extrastriate function. Now that we have established this new phenomenon, we plan to investigate the pharmacological and synaptic mechanisms that underlie the suppressive action of the corpus callosum.

Neural mechanisms for shape analysis in IT cortex

Anatomical experiments in our laboratory have shown that inferior temporal (IT) cortex is the last exclusively visual area in the cortical object recognition system, and our neurobehavioral experiments have shown that IT cortex plays an especially important role in the visual memory of objects.

In our first study of IT neurons, we surveyed their response to a large variety of stimuli, both simple and complex. We found that, like neurons in other visual areas such as V4, most IT neurons give at least a small response to many stimuli but respond better to some stimuli than to others. Presumably, therefore, the neural representation of objects in IT cortex is reflected in the pattern of activity across a population of cells and not in the activity of individual cells that respond only to specific objects.

In our survey of IT neuronal responses, we found that many cells seemed more sensitive to the overall shape of stimuli than to the location and quality of individual edges and contours. Therefore, in a subsequent study we examined how IT cortex might extract information about the overall shape of an object from information about its boundary. We adopted a method of representing shapes in terms of shape descriptors that have been used in computer pattern-recognition systems. The results were very encouraging in that over half the cells tested in IT were tuned to different shape descriptors. For two-thirds of the tuned cells, the shape of the tuning curve remained invariant over changes in the size of the stimulus and in its position on the retina. These results support the possibility that the visual system, and inferior temporal cortex in particular, use periodic shape descriptors in

classifying objects. Both our survey of IT neuronal responses and our investigation of shape selectivity are complete. Since many of our findings are being actively pursued by other laboratories, we will devote less effort to stimulus coding in IT cortex and more to other lines of investigation.

Functional architecture for motion perception in area MT

Area MT in the macaque appears to play less of a role in object recognition than areas V4 or IT but may play a greater role in spatial perception. Previously during the course of this project, we found that area MT contains a columnar architecture for analyzing the direction of stimulus motion. The representation of direction of motion in MT is strikingly similar to the representation of orientation in the primary visual, or striate, cortex. Even the size of the columnar systems is similar - 180 degrees of direction of motion (a motion 'module') in MT is represented within a piece of cortex 400 to 500 microns wide, the same size as the representation of 180 degrees of stimulus orientation in striate cortex.

Our results suggested that 0.5 mm wide 'modules' may be the fundamental unit of organization in extrastriate areas as well as in striate cortex. Yet, we were left with a puzzle. Although the cortical dimensions of the modules in MT are comparable to those of the modules in striate cortex, the total area of MT is much smaller. How could MT contain enough modules to analyze stimuli in all parts of the visual field? To answer this question, during the past year we examined the fine-grained visuotopic organization of MT. We found that the portion of the visual field analyzed by each module in MT was much larger than in striate cortex. Consequently, there appears to be a loss of resolution in MT - two stimuli in the visual field that activate separate modules of cells in striate cortex may activate the same module of cells in MT. How can the visual system extract from MT information about only one of the two stimuli? The solution to this apparent problem may come from our studies on selective attention, described next.

Neural mechanisms for attention and memory

Selective attention gates visual processing in extrastriate cortex

Our retinas are constantly bombarded by a welter of shapes, colors, and textures. Since we are aware of only a small amount of this information at any one moment, most of it must be filtered out centrally. Yet, this filtering cannot easily be explained by the known properties of the visual system. At each successive stage along the pathway from the primary visual cortex into the temporal lobe, the pathway known to be crucial for pattern perception, there is an increase in receptive field size. Many different stimuli will typically fall within these large receptive fields, and thus, paradoxically, more rather than less information appears to be processed by single neurons at each successive stage. How then does the visual system

limit processing of unwanted stimuli? The results of our single-neuron recording experiments in visual cortex of trained monkeys indicate that unwanted information is filtered from the receptive fields of neurons in extrastriate cortex as a result of selective attention.

The general strategy of the experiment was as follows. While the monkey held a bar and gazed at a fixation spot, a stimulus appeared briefly at one retinal location followed shortly by a second, briefly presented stimulus at the same location. The monkey was rewarded for releasing the bar immediately if the

two stimuli matched and for releasing the bar after a fixed delay if they did not match. At a second retinal location, two other, irrelevant stimuli were also presented on each trial, each concurrently with one of the stimuli used in the task. The animal attended to the stimuli at one location for a block of trials and then, after a cue, switched its attention to the stimuli at the other location for another block of trials. The location of the animal's attention was repeatedly switched back and forth. Thus, identical sensory conditions were maintained across trials, but the locus of the animal's attention varied.

We recorded from neurons in both area V4 and IT cortex in two rhesus monkeys and found that the locus of the animal's attention within a neuron's receptive field had a dramatic effect on the neuron's response. When both attended and ignored stimuli were within the receptive field of either a V4 or IT cell, the response of the cell was determined by the attended stimulus; the influence of the unattended stimulus upon the cell was greatly attenuated. For example, if a cell was selective for red stimuli, it would respond well if a red stimulus appeared at an attended location but poorly or not at all if a red stimulus appeared at an ignored location. Thus, we have shown for the first time that the processing of unwanted visual stimuli in extrastriate cortex can be blocked as a result of selective attention. Furthermore, it seems that focal attention may also allow the visual system to extract information about stimuli at specific locations, in spite of the very large receptive fields of cells in the extrastriate cortex.

One important difference we found between area V4 and IT cortex was in the retinal distance over which attention could exert its effects. In V4, a cell's response to an ignored stimulus was suppressed only if both the attended and ignored stimulus were located within its receptive field. In the central visual field representation of V4, the receptive fields were only 2° to 4° wide. By contrast, receptive fields of cells in IT cortex were so large that the responses of cells could be influenced by attention to stimuli throughout at least the central 12° of both the contralateral and ipsilateral visual fields (the maximum distance that could be tested).

The results from V4 and IT cortex indicate that the filtering of irrelevant information is at least a two-stage process. In V4, only those cells whose receptive fields encompass both attended and unattended stimuli will fail to respond to unattended stimuli. In IT cortex, where receptive fields may encompass the entire visual field, virtually no cells will respond well to unattended stimuli. It is presumably as a result of this two-stage process that we are able to identify and remember the properties of a particular stimulus out of the many that may be acting on the retina at any given moment.

The mechanism for selective attention

In subsequent studies we have investigated the mechanism by which attention gates V4 and IT neuronal responses and how this mechanism interacts with other non-sensory influences on the cells.

We first established that attention does not influence visual processing in stations prior to V4. Anatomical experiments in the laboratory indicate that

V4 receives its visual input from the primary visual cortex by way of area V2. Our recordings in both the primary visual cortex and V2 have established that selective attention has little or no effect on visual processing in these areas. Thus, there was no evidence that the effects of attention on neuronal responses observed in V4 were actually generated at a prior stage and simply passed on to V4.

An analysis of visual latencies in V4 and IT cortex indicated that the attention mechanism does not act chronically to suppress responses to stimuli at ignored locations, but rather is driven or triggered by the onset of the attended stimulus. The visual latency of the suppressive mechanism appeared to be 100 msec in V4, since shorter latency (75-100 msec) responses in V4 to ignored stimuli were unaffected until 100 msec after stimulus onset, at which point they were sharply cut off. Furthermore, if on a given trial the normally attended stimulus was not presented, the response to the normally ignored stimulus was not suppressed. Conversely, the presentation of novel stimuli can also negate the suppressive mechanism, since the responses to unexpected stimuli within the receptive field were not suppressed.

On the basis of the above results, we suggest that the neurons that suppress extrastriate responses to ignored stimuli are located outside of V4 and IT cortex, that they receive visual inputs either from V4 or in parallel with V4, that it takes at least 100 msec from the onset of a stimulus to suppress extrastriate responses, and that the attention mechanism itself can be switched by novel events. Anatomical experiments in the laboratory indicate that two possible sources of the suppressive inputs to V4 and IT are the pulvinar and the posterior parietal cortex. We are currently recording from neurons in these areas to determine if they play any role in the gating of extrastriate responses by attention.

Local Metabolic Mapping

The results from our neurophysiological recording experiments indicate that the neural mechanisms underlying object recognition are reflected not so much in the activity of individual neurons as in the distribution of activity across a large population of neurons. Local metabolic mapping with the 2-deoxyglucose technique, developed by Sokoloff in the Laboratory of Cerebral Metabolism, provides a powerful tool for investigating the distribution of activity over wide areas. We are using a modification of the Sokoloff technique, developed by S.J. Schein in the NEI, which has a resolution practically to the single neuron level. In collaboration with Dr. Schein over the past year, we have established procedures for using the Schein method in awake macaque and marmoset monkeys viewing a computer-generated computer graphics display. In addition, we have developed a method for reversibly paralyzing an eye of the monkey so that stimuli can be placed at precise locations on the retina of an awake animal. The marmoset is a particularly useful animal for these studies since it is nearly lissencephalic and its cortex can be flat-mounted. Thus, we can view the distribution of activity throughout its entire cerebral cortex in a single section. Over the next year, we will begin to study the effects of sensory coding, selective attention, and visual memory on the distribution of cortical activity.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The primate, including man, is a highly visual animal. Thus, it is not surprising that perhaps half of the primate cerebral cortex is devoted directly or indirectly to visual processing. Consequently, the study of neural mechanisms of vision is not only of fundamental importance for our understanding of visual perception and memory but also for our understanding of brain function in general. The extrastriate visual areas described in this project are of particular importance to the field of neurobehavioral research because they contain the neural mechanisms for visual memory, and they are the direct source of nearly all the visual information to the limbic affective and motivational systems.

PROPOSED COURSE OF RESEARCH:

Our findings that neurons in prestriate area MT are organized within direction-of-motion columns, that neurons in prestriate area V4 appear to separate figure from ground, and that neurons in inferior temporal cortex appear to code object shape all indicate that the study of single neurons can give us valuable insight into the neural mechanisms of perception. Within the past year, we have been encouraged that cognition itself may be explored at the single neuron level, since we have observed that unwanted information is actively filtered from the receptive fields of extrastriate neurons as a result of selective attention. Clearly we have only scratched the surface of these topics. Within the next year we will try to unravel some of the complex neural circuitry underlying selective attention, and we will begin to study the interaction of the mechanism for selective attention with the neural mechanisms for the separation of figure from ground and the recognition of shape. In addition, we hope to extend our single neuron analyses to a study of the cortical and subcortical mechanisms underlying recognition memory. Finally, utilizing newly developed local metabolic mapping techniques, we will study the functional architecture of the extrastriate cortex.

PUBLICATIONS:

Desimone, R., Albright, T.D., Gross, C.G., and Bruce, C. Stimulus selective properties of inferior temporal neurons in the macaque. J. Neurosci. 4: 2051-2062, 1984.

Gross, C.G., Desimone, R., Albright, T.D., and Schwartz, E.L. Inferior temporal cortex as a visual integration area. In F. Reinoso-Suarez and C. Ajmone-Marsan (eds.): Cortical Integration, Raven Press, New York, pp. 291-315, 1984.

Desimone, R., Schein, S.J., Moran, J., and Ungerleider, L. Contour, color and shape analysis beyond the striate cortex. Vis. Res. 25: 441-452, 1985.

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Desimone, R., Schein, S.J., and Albright, T.D. Neural mechanisms for form, color and motion analysis in prestriate cortex of the macaque. In C. Chagas, R. Gattass, and C. Gross (eds.): Pattern Recognition Mechanisms, Pontifical Academy of Sciences, Vatican City, pp. 165-178, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02037-04 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional anatomy of the somatosensory cortex of the monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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LAB/BRANCH

Laboratory of Neuropsychology

SECTION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A pathway by which somatosensory information could reach the limbic structures in the temporal lobe known to be critical for tactile memory has now been delineated. To trace this pathway, the anatomical connections of electrophysiologically identified somatosensory fields lying in or near the lateral sulcus of the macaque were investigated with both anterograde and retrograde axonal transport techniques. The data show that a series of parallel tactile processing pathways converge on the insular cortex; this region, in turn, projects directly to the amygdala and indirectly to the hippocampus via the rhinal cortex, thus linking the somatosensory cortices with the limbic structures of the temporal lobe.

To further describe the functional properties and relationships of the various somatic fields, electrophysiological studies have been undertaken. Neurons of the posterior insula have response properties typical of higher order processing fields in other modalities. Further, the somatic fields in the lateral sulcus appear to be dependent on SI for their somatic input because neurons in these fields no longer respond to tactile stimulation following ablation of SI. To examine this problem from a comparative perspective, the cortical connections of the somatic fields have been studied in the cat. The cat and monkey have similar connectional patterns, but, in the cat, there are more widespread connections among these fields.

The contribution of thalamic inputs to the functional properties of the somatic fields have been studied with axonal transport techniques. The findings shed new light on the organization of thalamocortical connections by showing that different thalamic nuclei project to the first (SI) and second (SII) somatic fields and that somatic fields outside of S1 receive their inputs from an array of thalamic nuclei, rather than just one, as had been thought previously.

PROJECT DESCRIPTION:

Work in this laboratory has shown that the amygdala and hippocampus are critical not only for visual memory but also for tactual memory. Though these studies suggested that the second somatosensory area (SII) and the insular cortex may act as relays for the transfer of somatic inputs from the first somatosensory area (SI) to the limbic system, there are a number of other somatosensory fields in or near the lateral sulcus that could serve this relay function. Because little is known about the connectivity or other properties of these fields, we have undertaken a study with the goal of delineating (I) the route via which somatosensory information reaches the limbic structures critical for memory, (II) the corticocortical interrelations of the somatic fields involved, (III) the thalamic relationships of these fields, (IV) their single-unit response properties, and (V) the sources of somatic input to the higher-order somatic processing areas. Further (VI), similar studies have been undertaken in the cat, in collaboration with R.S. Waters at Rockefeller University, in order to obtain data from a second species and also to examine the motor projections of somatic cortical fields.

Methods Employed:

Single-unit and multi-unit recording techniques were used to identify the specific cortical fields in the lateral sulcus of the macaque that are activated by somatic input. These fields include the second somatosensory area (SII), area 7b, the retroinsular area, and the granular and dysgranular insular fields. In early experiments, after a particular field was mapped, an injection of either tritiated amino acids (a mixture of proline and leucine) or HRP was made into the hand or digital representation within it to trace its connections. In later experiments, injections into the representations of other body parts have been made to help outline the projection zone of each field. Accurate placement of the injection was ensured by either (i) injecting through the recording pipette by iontophoresis or ii) recording from a microelectrode cemented to the needle of the injection syringe.

Histological identification of cortical fields has been improved through processing of adjacent sections to reveal either cell bodies, with a standard Nissl stain, or axons, with a sensitive silver stain we have developed for bulk use.

Preliminary physiological studies of lateral sulcus neurons have been performed in immobilized monkeys, which were lightly anesthetized, and more complete studies are being performed in awake monkeys seated in a primate chair.

In another group of monkeys the cortical fields comprising the first somatosensory area have been ablated in one hemisphere. In some of these animals the corpus callosum and anterior commissure were split and in others the commissures were left intact. Single-unit recording studies of SII and the granular insular field ipsilateral to the lesion were performed to determine if tactile stimulation of the body surface could drive units in these two fields in the absense of SI cortex.

In cats, somatosensory and motor fields were identified by microstimulation methods. The somatic fields SII, area 5, and SIV, along with primary and premotor cortex were then injected with HRP and tritiated amino acids to map the cortical and thalamic afferents and the cortical and brainstem efferents, respectively.

Major Findings:

I. Corticocortical Connectivity:

Using the combined recording-injection techniques described above, we have placed injections into SI, SII, area 7b, area 5, the retroinsular field (Ri), and the granular (Ig), dysgranular (Id), and agranular (Ia) insular fields. By combining the data concerning anterograde projections derived from the tritiated amino-acid injections and retrograde projections derived from the HRP injections, we have demonstrated reciprocal connections between: SII and Ri, SII and area 7b, SII and Ig and Id, and Ri and Ig. Also, we have confirmed previously reported reciprocal projections between SI and SII and demonstrated reciprocal projections between area 5 and both Ri and area 7b. Finally, anterograde labeling resulting from HRP injections into the insular fields has confirmed recently reported projections from Ig, Id, and Ia to the amygdala and from Id and Ia to the prorhinal and perirhinal cortical areas. These areas, in turn, are known to send projections to the hippocampus.

Our studies thus demonstrate that tactual information may reach the amygdala and hippocampus via relays in the granular and dysgranular insular fields, which receive their somatic cortical inputs from SII and Ri. A ventrally directed cortico-limbic pathway originating in SI may therefore be important for the perception and memory of somatosensory stimuli. This possibility will be examined directly in a series of neurobehavioral studies.

II. Laminar Patterns of Termination:

Three different laminar patterns of termination of the corticocortical projections described above were seen. Each pattern depended on the field into which the injection was made and the field with which the injected field was connected. Though similar patterns have been described in other areas of the cortex, only one of these patterns has previously been reported in the somatosensory system.

This pattern consists of a heavy band of labeled terminals in layer IV, with progressively lighter labeling, indicative of fewer terminals, in the supragranular layers, III, II, and I. There is a light band of terminal labeling in layer VI paralleling that seen in layer IV. This pattern has been described for the projections from SI to SII and area 5, and from area 5 to Ri and 7b. It is similar to the forward projections (i.e. projections outward from striate cortex) seen in the visual systems.

The second pattern is analogous to the one described in the visual system as a backward projection (i.e. one projecting towards the striate cortex). Its most striking characteristics are a complete absence of labeling in layer IV and heavy labeling in layer I. Additional labeling is seen in layer VI and

sometimes in layers III and II. The projections from SII to SI and Ri, from Ri to area 5, from Ig to SII and Ri, and from Id to SII are all of this type.

The third pattern, previously described in prefrontal association areas, consists of a single, apparently homogeneous column of labeled terminals extending from layer VI through layer I. Its most striking feature is the lack of laminar differences in labeling density, in sharp contrast to the so-called forward and backward projections described above. This pattern is seen in the projection from SII to area 7b.

By analyzing the pattern of forward and backward projections, we have been able to determine the probable sequential order in which information is processed in the somatosensory system. According to this analysis, the forward direction is SI to SII and area 5, area 5 to Ri and area 7b, area 7b and Ri to SII, and SII and Ri to Ig. SII also projects to Id. Ig and Id then project to the limbic system.

III. Thalamocortical Relations:

In conjunction with the foregoing work, the thalamic connectivity of the cortical fields of the somatosensory system was thoroughly examined. This study was required because preliminary findings indicated that the thalamic relations of the cortical fields described above differ from those described in the literature. By having, for the first time, an appreciation of the full extent of these fields, and by using our combined recording-injection techniques to increase the accuracy of our injections, we have been able to provide a new account of this fundamental anatomical relationship.

There are three major new findings: (1) The second somatosensory area (SII) receives its major thalamic input from the ventroposterior inferior thalamic nucleus (VPI) and additional inputs from the central lateral nucleus and the caudal division of the ventroposterior lateral nucleus (VPLc); the latter inputs, in contrast to previous reports, arise only from neurons widely scattered in VPLc and in the ventroposterior medial nucleus (VPM). The finding in the monkey that SI and SII receive different thalamic inputs is consistent with the hypothesis that they process information in a sequential rather than parallel manner, the latter notion having been based on previous reports that both fields received their thalamic input from one source, VPLc. Because of the importance of determining whether the cortical processing in SI and SII is sequential or parallel, we are pursuing this question with additional experiments (see V below).

(2) The cortical fields outside of SI receive their thalamic inputs from an array of thalamic nuclei rather than just one, as had been assumed previously. Thus, the dysgranular insular field (Id) receives thalamic input from the basal ventromedial nucleus (VMb), VPI, the posterior nucleus (Po), the suprageniculate (SG) and limitans (Li) nuclei, and the medial pulvinar (Pulm). The granular insular field (Ig) receives its input from SG, Li, Po, VPI, and Pulm. The retroinsular area (Ri) receives from Po, SG-Li, Pulm, and perhaps from the magnocellular division of the medial geniculate nucleus (MGmc) and VPI. Area 7b receives inputs from the oral and medial divisions of the pulvinar (Pulo and Pulm), the lateral posterior nucleus (LP), the caudal

division of the ventrolateral nucleus (VLc), and the parvocellular division of the medial dorsal nucleus (MDpc). SII, in addition to its inputs from VPI and VPLc, also receives projections from Po and SG.

Although each field thus receives multiple inputs, each does appear to be dominated by inputs from one nucleus. Thus, SII is dominated by VPI, 7b by Pulo and the immediately adjacent part of Pulm, Ri by Po, Ig by SG-Li, and Id by VMb. Examination of the patterns of retrogradely labeled neurons within nuclei suggests that the thalamus may be organized in a manner analogous to the striatum. In the striatum, patches of neurons with specific transmitter and receptor populations also have specific afferent and efferent connections. These patches are surrounded by a matrix of neurons with different populations of transmitters and receptors and with different afferent and efferent connections. In the thalamus, the identification of transmitters and receptors is not as complete as it is in the striatum, but the patterns of afferent and efferent connections suggests that individual thalamic nuclei, such as VPLc, may have a modified type of patch-matrix organization.

(3) Pulm projects to a number of the cortical somatosensory fields including Id, the granular insula (Ig), and area 7b. Thus, the cortical territory receiving projections from the medial pulvinar includes not only the frontal, parietal, and temporal lobes, as previously reported, but also the insula. The nature and functions of this widespread projection are still unknown.

These findings suggest a revision of the traditional view of thalamocortical organization, which states that a single thalamic relay nucleus projects to a single cortical field. In the somatosensory system, at least, it now appears that each cortical field outside SI receives an array of inputs from a number of thalamic nuclei and that each thalamic nucleus projects to several cortical fields.

IV. Electrophysiological Studies of Insular Cortical Neurons:

In our exploration of the neuronal properties of the granular insula (Ig) in awake monkeys, we recorded both single and multiple units. Over 500 individual recordings were made through a region extending from the retroinsular area caudally to the level of the arcuate sulcus rostrally. On histological examination, we identified 237 units as having been located in the posterior insula, Ig. Of these Ig units, a minority (32%) could not be driven by any of the stimuli tried in any sensory modality, while the remainder (68%) were driven by innocuous, somatic stimuli. The population of somatic units was composed of two subgroups, one activated by intraoral stimulation (21%) and the other by stimulation of the body surface (79%). Of the latter, 61% were driven by stimuli classified as cutaneous (light touch or stroking) and 39% by stimuli classified as deep (joint movement or pressure on deep structures). There was no convergence between the two submodalities, nor were any of the somatic units responsive to gustatory, auditory, or visual stimuli, though 7 of the body-surface units did respond more intensely when the animal appeared to attend visually to the stimulating object. A prominent characteristic of the body-surface units was bilaterality of their receptive fields (86%).

There were several characteristics that distinguished the anterior from the posterior portions of Ig. Anterior Ig contained most of the units responding to intraoral stimulation (78%) and to stimulation of the face (92%). Anterior Ig also contained 95% of the undriven units, 78% of the units with small receptive fields, and 70% of the units responsive to deep stimuli. The posterior portion of Ig contained all of the whole-body units, i.e. those responding to stimulation of almost the entire body surface. However, more units responding to parts of the body surface exclusive of the head were also found in the anterior region (64%). Additionally, in both regions, units responding to different body parts other than the head were often found adjacent to each other. Thus, there was only a rough somatotopy, with the majority of face units located anteriorly but with considerable convergence for all other parts of the body surface.

Because the units in the granular insula, like those in visual area TE, are modality specific and have bilateral receptive fields, our data are consistent with the proposal that Ig serves as a critical link in a somatosensory-limbic pathway much as area TE does for the visual-limbic pathway.

V. Electrophysiological studies of SII neurons in animals with SI ablations.

We have examined the responses of SII neurons to somatic stimulation in monkeys anesthetised lightly with nitrous oxide. In intact animals, SII neurons respond to light tactile stimulation of small, circumscribed receptive fields located predominantly on the contralateral side of the body. In animals chronically prepared with ablations of SI that spared only portions of the head and foot representations, the vast majority of SII neurons no longer responded to somatic input.

In two monkeys with SI ablation combined with forebrain commissurotomy, 50 penetrations were made into the region of the lateral sulcus in which SII is located. In those penetrations 179 single units or multiunit clusters were isolated and studied for their responsiveness to tactile stimulation. Units in three of these sites responded to tactile stimulation of the head or face,and eight responded to stimulation of the hand or arm. Of those responding to forelimb stimulation, only four responded to light tactile inputs. By contrast, in six normal animals in which recordings were made to locate sites for injections of anatomical markers, 109 out of 119 recording sites in SII responded to tactile stimulation. Of the 75 of these units that could be clearly classified as responding to either light cutaneous or deep stimulation, 55 responded to light tactile inputs. In a third monkey with SI ablation whose forebrain commissures were left intact, 43 of 129 recording sites had units that responded to tactile stimulation. The units in the rest of the sites could not be driven. Of the 43 responsive units only 19 responded to light tactile stimulation and only 4 of those were located on the hand or arm.

These data suggest that the input SII receives from SI is necessary for the responsiveness of SII units to light tactile stimulation. The results are thus consistent with the hypothesis, generated on the basis of the differential laminar patterns of termination of the reciprocal SI-SII projections, that the primary flow of information about sensory input is from

SI to SII and that these two fields, at least, are organized in a hierarchical manner. The results are also consistent with the thalamocortical evidence suggesting that SII does not receive a primary somatosensory input from VPLc.

VI. Studies on the projections of cortical somatic fields in the cat.

In order to obtain data concerning the organization of the cortical somatosensory fields in a second species, and to begin an examination of sensorimotor relations, injections of tritiated amino acids were made into areas 4, 5, and SIV of the cat. As a first step, projections from these cortical areas to the brain stem were examined. The results demonstrate a set of parallel pathways to several regions of the mesencephalon and pons.

After injections into area 4, the magnocellular portion of the red nucleus (RNmc), the intermediate and deep layers of the superior colliculus (SC), and the ventral portion of the periaqueductal gray were labeled in the mesencephalon. More caudally, label was found in the tegmental reticular nucleus, central tegmental fields, and vestibular nuclei. Area 5 injections produced labeling primarily in RNmc, with an additional, smaller projection to the intermediate layers of the SC. Injections into SIV labeled primarily the intermediate and deep layers of the SC, with additional labeling in the ventral pontine nuclei.

The patterns of labeling in the RN appeared similar after area 4 and area 5 injections, while the patterns of labeling in the SC appeared similar after area 4 and SIV injections. Thus, area 5 and SIV each appear to duplicate one portion of the motor cortical output arising in area 4.

Within the cortex, these fields are widely interconnected. Motor cortex projects to SII, area 5, and SIV. SII projects to motor cortex, area 5, and SIV. SIV projects to motor cortex, area 5, and SII. Area 5, by contrast, projects to motor cortex and SIV, but not to SII. These results demonstrate the existence of reciprocal connections between all pairs of related fields except SII and area 5. They also emphasize the intimate relations of the somatic and motor cortical fields.

Although individual cortical fields are connected to similar sets of fields in the monkey and cat, the laminar termination patterns seen in the monkey were not seen in the cat. In the cat, only one laminar termination pattern was observed. In this pattern, the densest and most extensive label was always in layer I. Column-like extensions of label descended through the rest of the cortical layers, but these extensions were always less dense and had a more limited horizontal spread than the label in layer I. Furthermore, there were no consistent laminar variations in the density of label in layers II-VI. This single pattern resembles the backward pattern described above for the monkey, in which the densest label was, again, in layer I. It differs from the backward pattern in that, in the monkey, layer IV contained no label, whereas in the cat, layer IV was as densely labeled as layers II, III, V, VI. Moreover, in the cat, we did not observe the forward pattern that is commonly seen in the monkey. There is, therefore, a fundamental difference in the cortical organization of the cat and monkey and the way in which cortical communication occurs in these two species.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Previous studies concerning the connections of the somatosensory system have been relatively restricted in scope. This project suplies the first comprehensive look at the entire somatosensory system and how it may connect with the limbic structures necessary for memory. Furthermore, this project is yielding fundamental insights into how the cerebral cortex processes information by describing the precise laminar pattern of connections and by adding new data about the thalamic connections of these fields. As a whole, our studies have demonstrated remarkable parallels between the organization of the somatosensory and the visual systems, suggesting taht common mechanisms of perception and memory operate within both, and that further studies of each one will illuminate the other.

PROPOSED COURSE OF RESEARCH:

Progress on this project has slowed due to loss of key personnel during the past year. However, a postdoctoral fellow has recently joined the laboratory, and so progress is expected to accelerate during the coming year.

The anatomical tracing experiments have now been completed and two papers describing the results have been submitted for publication. The physiological recordings of insular neurons will continue in order to expand our sample size and to examine more anterior portions of the insula. Additional animals will be prepared with SI lesions to replicate and extend our preliminary findings and to examine other somatosensory areas for their dependence on SI. Injections of tracers will be made into the thalamus to confirm the results obtained from the cortical injections described above.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02038-03 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ontogenetic development of memory and habit formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Bachevalier	Visiting Associate	LN NIMH
Others:	M. Mishkin	Chief	LN NIMH
	L.G. Ungerleider	Research Psychologist	LN NIMH
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COOPERATING UNITS (if any)

None

LAB/BRANCH

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Memory formation and habit formation are two qualitatively different retention processes based on separate neural mechanisms. On the evidence that the limbic memory system is not fully developed in infant monkeys, we have prepared monkeys with neonatal removal of this system to see how cognitive, emotional, and social behavior develops in animals whose infantile global amnesia might persist through adulthood. Animals with neonatal removal of area TE, a higher-order station of the visual system, serve as controls. The results so far indicate that, at two and six months of age, monkeys with neonatal limbic lesions display abnormal social behavior, whereas the operated controls are essentially unimpaired relative to normal infants. At three months of age, neonatal ablation of area TE leads to a transient impairment of habit formation (compared to permanent impairment seen with the same lesion in adults), whereas limbic lesions in both infants and adults leave habit formation intact. Interestingly, data on both normal and operated infants suggest that development of the habit system is sexually dimorphic, and that this is due to the high testosterone levels present in male infants before and shortly after birth. At ten months of age, the infants with limbic lesions show impairment in memory formation, whereas the operated controls show significant functional sparing (compared to those that received the same lesions as adults). Our tentative conclusion is not only that early and late brain damage have different consequences on learning and memory but also that the direction of the difference depends on whether the locus of injury is cortical or subcortical, the task measures habit or memory formation, and the subject is male or female. Although visual recognition memory measured by problem solving develops late in ontogeny, it can be demonstrated in early infancy when measured by the preferential-viewing task. Even though this type of recognition memory is a primitive process, it is nevertheless markedly impaired by either early or late limbic-system damage though not by neonatal ablation of area TE.

PROJECT DESCRIPTION:

Findings from studies of the effects of lesions in adult monkeys suggest that memory and habit formation are qualitatively different retention processes based on separate neural mechanisms. The memory system, which serves both recognition and associative memory, utilizes a cortico-limbo-diencephalic circuit. By contrast, the habit system, which mediates retention of stimulus-response connections, probably depends in large part on a cortico-striatal system. Our recent studies of behavioral development in infant monkeys have suggested that these two systems are developmentally dissociable, in that the nonlimbic habit system appears to mature considerably earlier than the limbic memory system. On the evidence that the limbic memory system is essentially nonfunctional in infants, we have prepared monkeys with neonatal removal of this system in an attempt to see how cognitive, emotional, and social behavior develop in animals whose amnesia might persist from infancy through adulthood. In addition, to evaluate whether visual recognition memory does indeed appear late in ontogenetic development, we have undertaken to measure the memory capacities of infant monkeys using a preferential viewing task, a task widely used to demonstrate recognition memory in human infants. In tandem with these developmental studies of behavior, we have begun to map the distribution of opiate receptor binding sites in the brain of a newborn rhesus monkey.

Experiment 1:

Infant rhesus monkeys received damage to either the limbic system (i.e. amygdalo-hippocampal complex) or the anterior part of inferior temporal cortex (i.e. area TE). The bilateral lesions were performed in two unilateral stages at approximately one week and three weeks of age, respectively. Each experimental and operated control animal was age-matched with a normal monkey. We are currently following the behavior of these animals from birth to five years of age in order to assess the effects of neonatally induced amnesia on (1) the maturation of cognitive functions and skill learning, as measured by a variety of visual memory, problem solving, and habit formation tasks, and (2) the development of emotional and social behaviors, as measured by interactions with familiar vs. unfamiliar and normal vs. operated monkeys of both sexes and various ages, and by reactions toward familiar vs. unfamiliar and emotionally neutral vs. emotionally challenging environments and stimuli. To date, we have prepared eight monkeys with bilateral amygdalo-hippocampal lesions and eight with bilateral TE lesions. These monkeys were age-matched with thirteen normal animals and have already undergone some testing for social behavior and learning abilities.

The results so far indicate that, at two and six months of age, monkeys with neonatal limbic lesions display abnormal social behavior, whereas the operated controls are essentially unimpaired relative to normal infants. At three months of age, neonatal ablation of area TE leads to a transient impairment in habit formation (compared to the permanent impairment seen with the same lesion in adults), whereas limbic lesions in both infants and adults leave habit formation intact. Interestingly, data from both the normal and the operated infants suggest that ontogenetic development of the habit system is sexually dimorphic, this system maturing earlier in females than in males (see

experiment 4). At ten months of age, the infants with limbic lesions are severely impaired in memory formation, whereas the operated controls show significant functional sparing of memory (compared to the animals given TE lesions as adults). These preliminary findings suggest that the consequences of early neural damage may be different from those of the same damage in adults and, further, that the direction of the difference depends on whether the locus of injury is cortical or subcortical, whether the subject is a male or a female, and whether the task measures habit or memory formation. The results suggest that early dysfunction of the limbo-thalamic memory system produces symptoms that are similar to the behavioral syndrome seen in autistic children. Both disorders are characterized by memory deficits, absence of social interactions, and development of ritualistic and compulsive behaviors, such as rocking and complex stereotyped movements. Although these preliminary results thus indicate that monkeys with neonatal limbic lesions might provide an animal model of autism, they are not conclusive inasmuch as we have not yet observed other symptoms characterizing autistic children, such as disinterest in initiating play when placed in a group, lack of separation anxiety, fearful responses to novelty, environmental complexity, and noises, dramatic emotional responses to change in routine, and unusual food preference. We therefore plan to retest the animals when they are 2-3 years old and analyze in detail their individual and social behavior for characteristic signs of autism. Even if these requirements for a valid model of autism were to be satisfied, there are still other questions to be examined. For example, the responsible pathology may be not combined amygdalo-hippocampal damage but amygdaloid damage only, since it is well known that such damage by itself is associated with changes in emotional and social behavior in adult monkeys. We are therefore currently adding animals with neonatal lesions of the amygdala alone and of the hippocampus alone to determine whether damage to only one of these two structures might produce the syndrome resembling autism or whether damage to both is necessary. To date, we have prepared two monkeys with amygdaloid lesions and two with hippocampal lesions. These monkeys were age-matched with two normal animals and have already undergone some testing for social behavior and learning abilities. The preliminary results indicate that, at two months of age, all four operated monkeys appear to be normal in their social behavior as well as in their performance on the visual discrimination task.

Experiment 2:

Our earlier findings on the development of memory formation in infant monkeys suggested that, as measured by problem solving, visual recognition memory develops late in ontogeny. To test the generality of that conclusion, we next traced the development of visual recognition using the preferential viewing task, a task widely used to demonstrate visual recognition in human neonates. In addition, to test whether the learning ability measured by this task is mediated by the memory system or the habit system, we have begun to assess the learning capacities of infants that have received neonatal temporal-lobe lesions.

Performance on the preferential viewing task, which measures how fixation time is distributed between a familiar and a novel visual stimulus, was assessed in the infants periodically at ages 5, 15, and 30 days and in adults at the age of 3-4 years. Like normal adults, the normal infant monkeys between 15 and 30

days of age gazed at the novel stimuli more than at the stimuli already gazed at several seconds earlier. Despite this evidence that this type of recognition memory is a primitive process, it was nevertheless markedly impaired by either early or late limbic-system damage. Since visual recognition as measured with problem-solving tasks in adults is also dependent on inferior temporal cortex (area TE), we tested whether removal of this cortical area in infancy, would, like neonatal limbic lesions, also produce an impairment. Surprisingly, even though the visual preference for novelty was completely absent in adults with bilateral area TE lesions, it was preserved in both the 15-day-old infants with unilateral area TE lesions and the 30-day-old infants with bilateral area TE lesions. These new findings support earlier data showing that visual recognition approaches adult levels even in early infancy and suggest further that, although area TE is involved in recognition memory in adults, neonatal removal of this cortical area leads to significant sparing of this function. As in Experiment 1, the results indicate that the consequences of early temporal-lobe damage on recognition memory differ from those of later damage depending on whether the locus of injury is cortical or subcortical.

Experiment 3:

Opiates have been shown to play a role in memory function, and the recent development of autoradiographic receptor-binding methods has indicated that, in adult monkeys, opiate receptors are distributed densely in structures intimately involved in memory processes. Because certain forms of memory develop late during maturation, we have started to trace the ontogenetic development of opiate receptor-binding sites in the infant monkey brain. In our first attempt, the distribution of opiate receptors in the brain of a newborn rhesus monkey was mapped by in vitro autoradiographic localization of [^{3}H] naloxone. The methods are outlined in another project from this laboratory (MH 02040). The autoradiographs at selected levels through the newborn brain were compared to those from two adult monkey brains that had been processed in the same way. Whereas the distribution of opiate receptors at birth appears to be adult-like in subcortical structures (both limbic and nonlimbic) as well as in allocortical areas, all neocortical areas, except the primary visual cortex, lack the laminar-specific patterns seen in the adults. We have now started to follow the developmental course of naloxone binding using infants at different ages, as well as map the distribution of other subtypes of opiate receptors.

Experiment 4:

As shown in Experiment 1, the ontogenetic development of habit formation is sexually dimorphic. Whereas female infant rhesus monkeys learned a concurrent visual discrimination task at about the same rate as both female and male adults, male infants are significantly slower in their learning. To pursue this finding, we have initiated a study to examine the neuroendocrinological substrate of the learning difference between males and females. In a pilot experiment, two newborn females and four newborn males were used, and two of the four males received orchiectomy at the age of 29 days. These six infants were then tested at the age of 3 months in a concurrent visual discrimination task. Immediately following behavioral testing, plasma testosterone levels

were measured. The preliminary behavioral results indicate that, whereas normal males required more trials to learn the task than did the normal females, the orchiectomized males performed at the same rate as the normal females. Although the results on the blood testosterone levels in these monkeys are not yet available, the data suggest that the high level of testosterone found in male infants before and shortly after birth could be responsible for their slower rate of learning and that modification of the level of testosterone early in life might have a direct influence on the maturation of certain structures within the system underlying habit formation.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Developmental studies of the effects of early brain damage are of great importance for the assessment and understanding of those errors of central nervous system maturation that cause children to become autistic, dyslexic, learning disabled, or mentally retarded. This project will supply the first comprehensive investigation of social and cognitive development of monkeys suffering from an amnesia induced early in infancy. It will thus permit comparisons of the cognitive and social behavior of the neonatally operated animals with those of animals who have sustained the same lesion in adulthood i.e. after memories have been formed and consolidated in cerebral tissue outside the limbic system. In addition, comparison of the effects of early cortical and subcortical lesions will help answer whether or not compensatory mechanisms always operate to promote recovery from early brain injury. Our preliminary results suggest otherwise. Finally, in assessing the effects of early and selective temporal-lobe damage on infant, juvenile, and adult behavioral patterns, this project will help to evaluate two provocative proposals from the clinical literature: (a) that early dysfunction of the limbo-thalamic memory system is one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology; and (b) that the reason a pure case of global anterograde amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clinical picture of an amnesic adult. Furthermore, the discovery that at least one type of recognition memory mediated by the limbic system is present neonatally provides new insight into the normal development of memory processes and indicates the need to identify further which memory processes become available to an infant at different points in its maturation.

PROPOSED COURSE OF RESEARCH:

Our goal is to continue examination of the effects of neonatal limbic lesions on social and emotional behavior as well as on memory and habit formation at several periods throughout development from infancy to adulthood in order to test whether such a preparation does indeed provide an animal model of childhood autism. With the discovery of an important sexual dimorphism in the development of the habit system, we plan to pursue studies aimed at determining the neuroendocrinological substrate of that dimorphism. We shall also pursue studies to determine how the learning capacities measured by preferential viewing differ from the learning capacities measured by problem solving. This will help to determine which capacities of the memory system

actually do appear late in ontogenetic development and, by implication, whether the phenomenon of infantile amnesia is indeed due to the absence of a fully functional memory system in early childhood. In addition, we shall continue our attempts to follow the development of opiate receptors in infant monkeys.

PUBLICATIONS:

Bachevalier, J. and Mishkin, M. An early and a late developing system for learning and retention in infant monkeys. Behav. Neurosci. 98: 770-778, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02039-03 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology of memory and habit formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evidence from patients with Alzheimer's disease suggests that the basal forebrain cholinergic system plays an important role in memory functioning. In support of this view, it was found that lesions of the basal forebrain in monkeys produce impairment in recognition memory and altered sensitivity to the effects of drugs that act on memory. Our results in normal monkeys show that compounds that interfere with cholinergic mechanisms, such as the anticholinergic agent scopolamine, produce impairments in recognition memory, whereas those compounds that enhance cholinergic activity, such as physostigmine, improve recognition memory. In addition, our results suggest that the anterograde effects of scopolamine on memory may be greater than its retrograde effects, implying an influence on storage rather than retrieval. In a separate study, we found that THC, the active ingredient of marijuana, impairs recognition memory but is without effect on habit formation even in high doses, a dissociation implicating the limbic system as the site of action of this drug.

PROJECT DESCRIPTION:

During the past year, we have continued our studies on the effects of peripherally administered pharmacological agents on memory and habit formation in monkeys. Our previous work suggested that differences in sensitivity to the effects of cholinergic drugs in normal animals and animals with lesions would provide valuable information concerning the functioning of the two learning systems. We are now attempting to further characterize these differences in sensitivity by a variety of experimental manipulations. These investigations of the effects of peripherally administered drugs are necessary before we can proceed to our next goal, which is to analyze the effects of injecting drugs directly into selected cerebral areas.

Experiment 1:

The basal forebrain cholinergic system consists of the nucleus basalis of Meynert, the medial septum, and the diagonal band of Broca. Together, these nuclei provide the major cholinergic input to the cortex, the amygdala, and the hippocampus. Dysfunction of this system has been proposed as one explanation for the memory impairments observed in Alzheimer's disease. In a previous study we found that neurotoxic lesions of all three nuclei in combination impaired recognition memory, whereas lesions of either the nucleus basalis alone or of the medial septum plus diagonal band alone were ineffective. These animals were then tested following the administration of the cholinergic receptor blocker scopolamine and the cholinesterase inhibitor physostigmine. The results suggested that, compared to unoperated controls, the animals with lesions of the nucleus basalis of Meynert were more sensitive to the interfering effect of scopolamine, whereas the animals with lesions of the medial septum plus diagonal band were less sensitive to the facilitating effects of physostigmine. Animals with lesions of all three nuclei appeared to be insensitive to the effects of either drug. Taken together, these results suggest that partial lesions of the basal forebrain cholinergic system alter the sensitivity of remaining cholinergic receptors to the effects of cholinergic drugs. Before pursuing this finding further, we are awaiting the results of a new series of studies aimed at characterizing the effects of the same cholinergic compounds on other forms of learning and retention in normal monkeys.

Experiment 2:

Our previous work showed that the anticholinergic agent scopolamine (SCOP) impairs visual recognition in monkeys performing a delayed nonmatching-to-sample (DNMS) task with trial-unique objects. To test whether the drug has a retrograde effect equivalent to its anterograde effect, we administered SCOP to three rhesus monkeys either before (task 1) or after (task 2) the acquisition phase of DNMS. During the acquisition phase, the animal was shown a series of 40 sample objects at the rate of 1 every 30 seconds. During the test phase, each sample object was paired with a novel object, and the animal was rewarded for choosing the novel object. During the test phase, the order of presentation was reversed (i.e. the last sample object in acquisition was the first to be paired with a novel object and vice versa). In task 1, the retention interval ranged from 1 to 40 minutes. In task 2, in order to allow

for testing retrograde effects, a 20 minute interval was imposed between acquisition and test, and, consequently, the retention interval ranged from 20 to 60 min. SCOP was administered 20 min. before the start of acquisition in task 1, and immediately after acquisition in task 2. SCOP doses of 10 and 17.8 ug/kg were tested on task 1 and doses of 10, 17.8, and 32 ug/kg were tested on task 2. On both tasks, linear forgetting curves were obtained. In task 1, 10 ug/kg of SCOP produced a marginal impairment and 17.8 ug/kg produced a significant impairment. In task 2, in which SCOP was administered after acquisition, neither 10 nor 17.8 ug/kg produced an effect, impairment being observed only with the highest dose, 32 ug/kg. The results suggest that SCOP has mainly an anterograde effect on recognition and hence probably affects storage more than retrieval. Furthermore, in each task, the effective dose of SCOP produced a forgetting curve shifted downward from but parallel to the nondrug control curves, suggesting that the anterograde effect of SCOP is equivalent to reducing the strength of the memory trace by increasing the passage of time following acquisition.

Experiment 3:

In a related study, we have examined the effects of SCOP on another form of memory, object-reward association. The amygdala, which is known to be especially important for this form of memory, receives its cholinergic input mainly from the nucleus basalis of Meynert. Studies which have examined the firing pattern of cells in the nucleus basalis have reported that a significant percentage of these cells are maximally responsive to either the presentation or termination of reward. Based on these two pieces of information, we hypothesized that a one-trial object-reward association task might be more sensitive than a recognition task to manipulations of cholinergic system activity. SCOP (10 and 17.8 ug/kg) was found to impair performance on this task, but the degree of impairment was essentially the same as that observed with the recognition task, the forgetting curves having been shifted downward from the nondrug control curves by a similar amount. Although the results thus indicated that an object-reward associative memory task was not more sensitive to cholinergic disruption than a simple recognition task, they provided strong additional evidence for a cholinergic contribution to memory.

Experiment 4:

Previous work from our laboratory has shown that memory and habit formation are qualitively different retention processes based on separate neural mechanisms. In a preliminary study conducted last year, we tested the effects of SCOP on 24-hour concurrent learning, a sensitive measure of habit-formation ability. In this task, the animal is shown a series of 20 object pairs once very 24 hours. The early results indicated that doses of SCOP that impaired recognition (10 and 17.8 ug/kg) did not impair habit formation. However, in new studies conducted this year, we have found that higher doses of SCOP (32 ug/kg) do impair the rate of learning on this task. These results suggest that cholinergic mechanisms may be involved in both memory and habit formation. At this time we are unable to say whether the dual effect of SCOP reflects an action on cholinergic neurons that are common to both learning systems, or whether it reflects instead a simultaneous action on separate sets

of neurons, each set belonging to just one system. The latter seems more likely, given the markedly different doses at which the effects appear.

Experiment 5:

In addition to these studies on cholinergic mechanisms, we have continued the investigation being performed in collaboration with the National Institute of Drug Abuse on the cognitive effects of delta-9-tetrahydrocannabinol (THC), the active constituent of marijuana. To test whether THC may be affecting cognition through an action on the limbic system, we investigated the effects of orally administered THC on the two learning abilities described above. One group of monkeys was trained on delayed nonmatching-to-sample, the test of recognition memory known to be dependent on the limbic system, and the second group was trained on 24-hour concurrent learning, the test for habit formation which is known to be independent of the limbic system. THC significantly impaired delayed nonmatching-to-sample performance, the scores decreasing to 80% of control levels following a 4 mg/kg dose (the largest dose examined on this task). In contrast, no significant impairment was found on concurrent learning, even with doses as high as 16 mg/kg. These results suggest that THC interferes with recognition memory more than with habit formation, and this in turn may reflect a selective action of THC on limbic structures.

Experiment 6:

Unlike the substrate of the memory system, which is known to include the limbic lobe and the medial diencephalon as major components, the substrate of the habit system is still unknown, although previous neurobehavioral studies from our laboratory have suggested that the neostriatum may be involved. To determine the contributions of this system to habit formation, we are studying the 24-hour concurrent learning performance of monkeys following administration of 1-methyl-1,2,5,6-tetrahydropyridine (MPTP), a recently discovered neurotoxin which selectively destroys the nigrostriatal dopaminergic system. When given in high doses, this compound produces an impairment in motor function similar to that observed in Parkinson's disease. MPTP is being given to two monkeys in a series of small doses, such that any effects on learning can be identified prior to the onset of motor-system dysfunction. Followng each dose of MPTP, the animals are tested for their ability to learn a new set of object discriminations. Although no impairment has been seen following five administrations of the drug, the total amount of drug administered to date does not yet equal the amount known to impair motor function.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Our results to date provide convincing evidence that cholinergic mechanisms play an important role in memory processes in monkeys. The use of the cholinergic receptor blocker scopolamine has been shown to be a useful tool for investigating these processes in normal animals. Furthermore, our results of testing cholinergic agents in animals with lesions of the basal forebrain cholinergic system suggest that these drugs may unmask losses of function that would otherwise escape detection. These results have important implications for drug therapy in Alzheimer's disease for they suggest that simply

increasing cholinergic activity may not be a rational therapeutic approach. Our continuing studies with THC document the deleterious effects on memory of this commonly abused drug and suggest an underlying communality in the site of action of both THC and cholinergic agents, namely, the limbic system.

PROPOSED COURSE OF RESEARCH:

We will continue to evaluate the differential effects of both cholinergic and noncholinergic compounds, including chronic high doses of THC, on memory and habit formation in normal animals as well as in animals with chemically induced lesions of discrete brain regions. In addition, we plan to initiate new studies in which drugs are injected directly into targeted brain sites while the animals are being tested for memory and habit formation in an automated apparatus.

PUBLICATIONS:

Aigner, T. and Mishkin, M. Effects of physostigmine and scopolamine on recognition memory in monkeys. Behavioral and Neural Biology (in press).

Aigner, T., Mitchell, S., Aggleton, J., Struble, R., Wenk, G., DeLong, M., Price, D., Mishkin, M. The effects of scopolamine and physostigmine on recognition memory in monkeys following ibotenic-acid lesions of the nucleus basalis of Meynert. Journal of Neuroscience (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02040-02 LN

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional analysis of neurotransmitter systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurotransmitter systems have been implicated in many higher-order functions, both cognitive and emotional, but specific sites and mechanisms of action have not been identified. Studies to localize specific receptors for certain neurotransmitters in the monkey have been undertaken to attack these problems. Findings following lesions of the amygdala suggest that there may be opiatergic projections from the amygdala to higher-order sensory processing areas, such as the anterior insula and orbitofrontal areas. Developmental studies show that whereas limbic cortical areas and most subcortical regions have adult-like opiate receptors at birth, neocortical areas have simplified and undifferentiated binding patterns unlike the patterns seen in adults. Metabolic studies link the level of mu opiate receptors to the rate of protein phosphorylation in the F1 band. Since phosphorylation of F1 protein has been correlated with learning, these results suggest that opiates may help control the learning-related phosphorylation process.

Autoradiographic localization of benzodiazepine and beta-carboline receptors in monkey shows that both drugs bind with apparently identical distributions, implying that they both act on the same brain regions to produce their effects on anxiety.

Autoradiographic localization of nicotinic and muscarinic cholinergic binding sites in monkey suggests that, in the cortex, nicotinic sites are situated so as to modulate incoming afferent information, whereas muscarinic sites are more likely to modulate intracortical processing.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT DESCRIPTION:

The limbic and diencephalic areas found in other projects of this laboratory to be critical for learning and memory contain high levels of opiate receptors, and it is now known that opiates may alter learning ability under a variety of circumstances. The pathways by which opiate-containing neurons may project to the cortex are still unknown, however, as are the specific pre- and post-synaptic effects of endogenous opiates. In addition, the limbic structures of the brain are known to be rich in receptors for nonopiatergic agents affecting emotions, such as the anxiety-inducing drugs, as well as for other agents important in learning and memory, such as the cholinergic drugs. The initial stages of this project will attempt to (I) describe the opiatergic pathways that may affect learning, (II) relate the level of opiate-receptor binding to learning-related protein phosphorylation, (III) describe the distribution of opiate receptors in developing brain that may account for the developmental increases in perceptual and cognitive ability, (IV) examine the distributions of receptors for benzodiazepines and beta-carboline that may account for the action of these drugs in decreasing and increasing anxiety, and (V) describe and compare the distributions of nicotinic and muscarinic cholinergic receptors that may represent the site of action of acetylcholine in memory and learning.

Methods Employed:

Receptor binding and in vitro autoradiographic techniques are used to localize and quantify receptors. After sectioning unfixed monkey brains on a cryostat, the sections are thaw-mounted onto slides and incubated in tritiated ligands for various receptors and processed for autoradiography. Metabolic studies are performed on homogenized monkey brain tissue that has been dissected according to functional and cytoarchitectonic criteria and then assayed for receptor-binding levels and for the rate of learning-related protein phosphorylation. Correlations are then performed between the levels of mu-receptor binding and protein phosphorylation.

Major Findings:

I. Opiatergic Projections of the Amygdala

Analysis of [^{3}H] naloxone binding levels has been performed on sections from one monkey brain that had received a unilateral amygdalectomy 30 days prior to sacrifice. The sections show an increase in naloxone binding ipsilateral to the lesion in the anterior portions of the insula, which projects to the limbic areas of the temporal lobe, and in regions of the orbitofrontal cortex that appear to be required for memory formation. This increased binding is interpreted as a denervation supersensitivity of mu receptors following amygdalectomy, and suggests that the amygdala sends an opiatergic projection to the cortical regions mentioned. We are now attempting to replicate this finding in additional monkeys, to extend the analysis to other regions of the brain, and to examine kappa and delta as well as mu receptor subtypes.

II. Metabolic Studies

In order to relate the levels of opiate binding with rates of learning-related

protein phosphorylation, correlations of naloxone binding levels and protein phosphorylation rates have been carried out in two monkeys. The results demonstrate correlations across 22 cortical areas between the density of [^{3}H] naloxone binding sites and the in vitro phosphate incorporation into four proteins. To relate the findings more closely with plasticity within the brain, visual cortical fields from striate to inferior temporal cortex were examined. Two of the proteins, a 51Kd, PI 4.5, and an 81 Kd, 4.0 PI, showed strong correlations with the levels of naloxone binding and with each other along this sensory processing gradient. Both proteins are substrates for kinase C and are stimulated by calcium. The phosphorylation rates of the two other proteins, which are cAMP dependent, did not show the strong correlations with levels of naloxone binding or with the rates of phosphate incorporation into the 51 and 81 Kd proteins. These findings suggest a local control of protein phosphorylation in monkey cerebral cortex corresponding to opioid receptor levels and indicate that opioid peptides may help control metabolic processes at the synaptic level. These findings may be related to processes that underlie memory formation because the 51 Kd protein is very similar to a protein in the rat, called F1, which has been implicated in the formtion of memories and in the changes that accompany long-term potentiation. F1 and the 51Kd proteins have similar 2-D gel PAGE patterns, the same PI, and are only 3 Kd apart in molecular weight. These findings suggest that a protein already related to memory formation in the rat may have an analog in the primate, and that this analog changes its behavior along the sensory processing gradient of the cortical visual system.

III. Developmental Studies

Because certain forms of memory develop late during maturation, we have started to trace the development of opiate receptor binding sites in the brains of infant monkeys. In our first attempt, the distribution of opiate receptors in the brain of a newborn rhesus monkey was mapped by in vitro autoradiographic localization of [^{3}H] naloxone. The results, which are described fully in another project from this laboratory (MH 02038, Experiment 3), show that whereas subcortical and limbic structures have adult-like patterns of naloxone binding, neocortical areas have an immature pattern. This pattern is nearly identical across all neocortical areas and is characterized by a lack of laminar-specific distribution of receptors seen in adults.

IV. Localization of benzodiazepine and beta-carboline binding sites

Although biochemical evidence suggests that benzodiazepine (BDZ) and beta-carboline (BC) binding sites are part of the same supramolecular complex, there has not yet been an anatomical demonstration of this receptor localization. To gather such evidence, we performed an in vitro autoradiographic mapping study of the distribution of [^{3}H] flunitrazepam (FLN) and [^{3}H] BC binding in a rhesus monkey brain.

The major finding is that, in the monkey, specific FLN and BC binding sites have apparently identical distributions. Limbic structures such as the amygdala and the hippocampus, which have been implicated in the mediation of anxiety, were differentially labeled in identical fashion by the two ligands.

The lateral, accessory, and cortical nuclei of the amygdala were heavily labeled by both. Also, there was intense labeling by both in the molecular layer of the hippocampus and in regions related to the hippocampus, such as the medial portion of the medial mamillary nucleus and the lateral dorsal and anterior nuclei of the thalamus. Furthermore, the subfields of the hippocampal formation could be distinguished on the basis of differential labeling densities of both ligands. By contrast, the locus coeruleus and the raphe nuclei, which have also been implicated in anxiety, could not be identified on the basis of labeling densities of either ligand.

The apparently identical patterns of the BDZ and BC binding suggest that these two agents are acting on the same, or closely linked, receptors. The location of especially dense labeling in certain limbic strctures supplies additional evidence that these structures are involved in the modulation of anxiety.

V. Localization of nicotinic and muscarinic cholinergic binding sites

Recent evidence linking the dementia of Alzheimer's disease with a specific loss of cholinergic markers in brain has ignited interest in the cholinergic systems of the brain (See also project MH-02039). Despite the rapid advances in mapping out these systems with immunohistochemical and autoradiographic techniques, the distribution in the monkey brain of the two major subtypes of cholinergic receptors is not completely known. We therefore undertook to map both nicotinic and muscarinic sites in adjacent sections of two monkey brains. [^{3}H] nicotine was used to reveal nicotonic receptors, whereas [^{3}H] quinuclidinyl benzilate was used to reveal muscarinic sites. The distributions of these sites have been analyzed in the cortex and thalamus.

Both nicotinic (N) and muscarinic (M) receptors were found in all cortical regions, but M receptors were more widely distributed across the layers of a given field and had a wider variety of laminar labeling patterns across different fields than N receptors. N receptors were restricted to a band in layer III. This band was densest and widest in the primary sensory areas (A1, S1, and V1), but no other variations in pattern were apparent. The most common pattern for M receptors consisted of a dense band of label extending from layer I through upper layer III, a receptor poor band in lower layer III, and another band of receptors, less dense than the supragranular band, in the lower layers. Other laminar patterns of M receptors ranged from an apparently homogenous distribution across all the cortical layers in orbital frontal cortex, to variations of the bilaminar pattern described above in S1 and V1, to a single dense band in layer III of A1. Only in A1 did the N and M patterns appear similar.

The laminar patterns of distribution of both N and M receptors described here for the monkey differ from those described previously in the rat. Nonetheless, in both species, N receptors are located in either layers III or IV, the targets for incoming afferent fibers, suggesting a role for nicotinic regulation of this input. M receptors, by contrast, are densest in the upper and lower cortical layers, a distribution consistent with a role for muscarinic modulation of intracortical processing.

Nicotinic receptors were densest in all three anterior thalamic nuclei (AM,

AV, and AD). In addition, the medial dorsal (MD), lateral dorsal (LD), lateral posterior (LP), pulvinar, and thalamic reticular nuclei contained moderately dense labeling. The lateral (LG) and medial (MG) geniculate nuclei and the entire ventral group contained somewhat less label, and within this group the least heavily labeled nuclei were those of the ventrobasal complex (VPL and VPM). By contrast, the habenular nuclei, the intralaminar nuclei, and the midline nuclei were conspicuous by their lack of label. Muscarinic receptors, like nicotinic receptors, were dense in the anterior nuclei, but in MD, the parvocellular division was more densely labeled than the magnocellular division, which contained little label. The pulvinar and LD also contained moderately dense labeling, but LP, like the entire ventral group, was less densely labeled. LG and MG again contained moderate amounts of label and the intralaminar nuclei, again had little, if any, label. The midline and thalamic reticular nuclei on the other hand, again had a moderate amount of label, as did the lateral habenula, which was not labeled by[^{3}H] nicotine.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Knowledge about the functions of neurotransmitter systems has both basic and applied value. The studies described here represent one of the few attempts to study these systems in primates. Descriptions of the opiatergic pathways of the forebrain will supply the fundamental descriptive information needed to understand the role of opiates in higher-order behavioral functions and will help guide neurobehavioral experiments by supplying targets for lesions. The studies of protein phosphorylation may supply information concerning the mechanisms by which the opiates affect neuronal metabolism in general and learning specifically. Eventually, this may lead to therapeutic advances in the treatment of memory and learning disorders. Localization of benzodiazepine and beta-carboline binding sites will supply descriptive information suggesting where their effects on anxiety may be mediated. Localization of cholinergic receptors will supply descriptive information and provide clues as to the sites and modes of action of cholinergic systems that modulate learning and memory.

PROPOSED COURSE OF RESEARCH:

Our major goals for the coming year are to replicate the initial findings and to extend them by looking at receptor subtypes. Specific aims for each of the five experiments are as follows: (I) examine additional brain regions that receive amygdaloid inputs and examine changes in mu, kappa, and delta receptor binding by using ligands that are highly specific for each; (II) further characterize the F1 protein by means of 2-dimensional gels, examine the activity of the specific kinase that phosphorylates F1, and quantify the F1 content in various regions of the monkey brain; (III) replicate the finding in the neonate with an additional animal and examine the distribution of mu, kappa, and delta receptors; (IV) replicate and quantify the initial findings on benzodiazepine and beta-carboline binding and determine whether amygdalectomy has an effect on such binding; (V) map the distribution of muscarinic and nicotinic cholinergic receptors in infant rhesus monkey brain.

Because the principal investigator has left the laboratory and become a guest researcher, progress on this project has slowed substantially. Goals not completed last year, therefore, have become goals for the coming year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00471-30 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Heredity and Environment in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Allan F. Mirsky, Ph.D. Chief LPP, NIMH

COOPERATING UNITS *(if any)*

Institute for Research on Kibbutz Education, Haifa University, Israel; Hebrew University, Israel; Oranim Teacher's College, Israel; Bar Ilan University, Israel; University of Chicago, Illinois; William Beaumont Hospital, Michigan

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome; (2) Studies of Danish adoptees and their biological and adoptive families; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel. We maintain contact with the quadruplets but have not pursued active studies with them during the past two years. The Danish adoptees are of continuing interest to us and we are preparing additional reports on factors involved in their psychiatric outcome. The Israeli children are the subject of intensive research efforts and we are planning further behavioral and biological studies with them.

PHS 6040 (Rev. 1/84) GPO 904-917

Others:

Edward K. Silberman, M.D.	Guest Researcher	LPP, NIMH
Shmuel Nagler, Ph.D.	Research Psychologist Institute for Kibbutz Education	Israel
Shaul C. Sohlberg, Ph.D.	Clinical Psychologist Bar Ilan University	Israel
Sol Kugelmass, Ph.D.	Professor of Psychology Hebrew University	Israel
Joseph Marcus, M.D.	Professor of Child Psychiatry University of Chicago	Chicago, Illinois
Judith Shotten, M.A.	Psychiatric Social Worker Hebrew University	Israel
Moshe Ayalon, M.A.	Oranim Teacher's College (deceased)	Israel
Loni Bonwitt	Oranim Teacher's College	Israel
Eugene P. Tassone	Psychologist	LPP/NIMH
Olive W. Quinn, Ph.D.	Guest Researcher	LPP, NIMH
Patricia Lowing, Ph.D.	Staff Psychologist William Beaumont Hospital	Michigan

Project Description

The project is composed of the following studies: (1) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. We are continuing our contacts with this family to see what happens in the clinical course of these women and to see how the course is related to earlier and to current life experiences; (2) Studies of adoptees and their biological and adoptive families in Denmark; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

1. The Genain Quadruplets

A series of three studies with first authors respectively, Lynn Delisi, Monte Buchsbaum and Allan F. Mirsky appeared in Psychiatry Research in 1984. These publications summarize an extensive series of behavioral, biochemical, neurophysiological, and neuroradiological tests conducted on the Genain quadruplets during a visit to NIMH during 1981. The procedures included: an extensive series of genetic identity tests; biochemical determinations from blood, urine, and cerebrospinal fluid of various catecholamine compounds with emphasis on dopamine and norepinephrine; procedures related to the identification of possible preexisting viral infection of the central nervous system; neuroradiological and neurophysiological tests (CT scan, PET scan, evoked potential and EEG brain maps, brain stem evoked potentials); and an exhaustive battery of psychological and psychometric tests with a special focus on measurement of attention, arousal and memory. Two of the tests were

essentially identical to measures employed in the late 50's--the continuous performance test and the reaction time paradigm. Further, for most of the behavioral tasks, we were able to examine the Genains both on and off medication--the latter after a period of at least two weeks free from the phenothiazine drugs they were taking on admission to the NIMH.

With respect to the varying degrees of illness seen in the Genains, the following findings appear relevant: the tests indicate that two of the women (Nora and Hester) deteriorated rapidly when removed from medication, and two (Iris and Myra) did not. The consequence of this is that the grouping of the quadruplets on the basis of their characteristics and abilities while they are medicated is different from that apparent while they are off medication. On medication the apparent pairing is Nora and Myra and Hester and Iris. Scrutiny of the test material, including the biochemical, physiological, neuroradiological and immunogenetic, as well as behavioral, leads to speculation that certain unique biochemical findings and differing types and amounts of cerebral pathology may constitute the fundamental cause of the variable expression of schizophrenia in the Genains. This set of circumstances is superimposed on a basic schizophrenic diathesis which is manifest in the biochemical and certain neurological and neurobehavioral findings. The interdisciplinary research effort represented by this series of studies is unique in the annals of schizophrenia research.

2. The Danish Adoptee Study--Reanalysis of the Data

Using data from Danish health records, in a now-classic study, Rosenthal, Kety and Wender compared the frequency of schizophrenia spectrum disorders in two groups of persons adopted in infancy or early childhood: those with a psychotic parent (index group) and those whose biological parents had never had psychiatric treatment (control group). Significantly more disorder was found in the index than the control group. We currently have under editorial review in the American Journal of Psychiatry an analysis of reported stress factors during the childhood of these Danish subjects which appear to relate to the severity of outcome in the schizophrenia spectrum.

3. The Israel Kibbutz--High Risk Study

During the past year, the Laboratory has published in the Schizophrenia Bulletin a report of work begun in 1962 on the study of children at risk for schizophrenia in Israel, which was designed and initiated by David Rosenthal. The study has examined 100 children, of whom 50 had one schizophrenic parent, and 50 were born to two nonschizophrenic parents. Half of both "index" and control groups were reared in towns in traditional nuclear families, while the remaining half were reared in communal settings on kibbutzim.

In broad outline, the results indicate that index children were discriminable from controls in many areas of function, but kibbutz and town children did not differ on the experimental examinations. Furthermore, kibbutz versus town rearing had no discernible effect on the performance or behavior of high-risk children. Index children were found to be poorer in psychosocial adjustment, perform more poorly in school, manifest a number of

neurological "soft signs," and show deficits on psychological tests requiring high levels of attention, visual integration, and visuomotor coordination. An important negative finding was lack of differences between index and control children on psychophysiologic measures of arousal and habituation in the first examination.

We have also conducted follow-up interviews with the study subjects, who are now in their mid-twenties, at the peak of their risk period for schizophrenic breakdown. Ninety of the surviving 99 subjects have been seen. Results show that nine subjects fall within the "schizophrenia spectrum" (of whom six are DSM-III schizophrenic), six from kibbutz backgrounds, and three from towns. When all DSM disorders are considered, more than five times as many ill subjects fall within the index (N=23) than within the control group (N=4). Furthermore, when schizophrenia itself is excluded, the remaining subjects with history of illness (including DSM-III Major Affective Disorder or Dysthymic Disorder) are found predominantly in the index-kibbutz cell (16 of the total of 23 in the cell, including 9 with affective disorder). Other significant preliminary results include persistence of attention-related deficits in the index group, and continued poor social and work adjustment in high-risk subjects.

We are currently planning to reexamine the hospital records of the parents of the Israeli cohort to attempt to uncover factors which may have led to the excess of affective illnesses in the offspring.

Significance to Biomedical Research and to the Program of the Institute

The issue of the mode of heritability of mental illness, and factors which modify it, may be the highest priority of the Institute. This work contributes significantly to our knowledge in this area and ultimately, to our capacity to treat and prevent schizophrenia and related disorders.

Proposed Course

We are in the process of planning a comprehensive reexamination of the subjects between 1985 and 1987. The new efforts will focus on factors which may have led to the excess of affective disorders (as noted above), but also include a repeat series of diagnostic psychiatric interviews and a series of biological, psychological, and physiological measurements. We are also attempting to obtain the complete military health records on the subjects, so as to ascertain whether breakdown began to occur during this period of the subjects' lives.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00484-25 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychophysiological Responsivity and Behavior in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Socio-Environmental Studies, Child Psychiatry Branch, Laboratory of Clinical Science, Neuroscience Branch, Biological Psychiatry Branch, and Clinical Neurogenetics Branch, NIMH; Hypertension-Endocrine Branch, NHLBI.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.8	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general purpose of this project is to investigate the roles of autonomic nervous system (ANS) activity, attention, and information processing and their interrelationships in the pathology, etiology, and prognosis of psychiatric disorders. A second purpose is to determine biological and psychological processes related to ANS activity and attention. ANS activity is assessed by peripheral measures, such as skin conductance, heart rate, and skin temperature. Subjects are tested under conditions of rest, presentation of tones, and performance on tasks such as reaction time and two-flash discrimination.

Biological mechanisms are investigated by correlating these variables with enzyme activity, neuropeptides, and levels of biogenic amines and their metabolites.

Studies are being done on unmedicated patients with diagnoses of schizophrenia, affective disorder, obsessive compulsive disorder, anxiety-panic disorder, and autism to test the diagnostic specificity of patterns of ANS activity. Effects of state changes are studied in cases of multiple personality, as well as with brain dysfunction as revealed by CT and PET scans. In some studies blood samples are taken during ANS recording sessions in which stressful procedures are given. In one, the effects of success and failure to escape an aversive noise are assessed, and in another, the effects of a dose of yohimbine is being studied. Clinical trials of various treatments are studied. These include pimozide, propranolol, verapamil, and hemodialysis in schizophrenia, clorgyline and clomipramine in obsessives, and alprazolam and imipramine in panic-anxiety patients.

Psychological correlates are studied via clinical background data, clinical ratings and questionnaires, and by procedural variations. The use of confirmatory factor analysis in data reduction and to improve quantification of ANS activity is being explored.

Others:

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Carmi Schooler, Ph.D.	Senior Investigator	LSES, NIMH
Dennis Murphy, M.D.	Chief	LCS, NIMH
David Pickar, M.D.	Chief	SCS, NSB, NIMH
Thomas Uhde, M.D.	Staff Psychiatrist	BPB, NIMH
Daniel Hommer, M.D.	Staff Psychiatrist	NSB, NIMH
Judith Rumsey, Ph.D.	Staff Fellow	LCS, NIMH
Judith Rapoport, M.D.	Chief	CHP, NIMH
Thomas Insel, M.D.	Research Psychiatrist	LCS, NIMH
Frank Putnam, M.D.	Staff Psyciatrist	NPB, NIMH
John Nurnberger, M.D.	Medical Officer	CNG, NIMH
Alan Breier, M.D.	Clinical Associate	NSB, NIMH
Margot Albus, Ph.D., M.D.	Guest Researcher	NSB, NIMH
David Goldstein, M.D., Ph.D.	Staff Fellow	IRHE, NHLBI
Joseph Zohar, M.D.	Visiting Associate	LCS, NIMH
Alec Roy, M.D.	Visiting Associate	NSB, NIMH

Project Description

A. Objectives

The major objective of this project is the further understanding of the role of autonomic nervous system (ANS) activity, information processing and attention, and their interrelationships in psychiatric disorders, primarily schizophrenia. The overall strategy involves studies of ANS and attentional relationships to diagnosis and prognosis, studies of the effects of drugs and other therapeutic interventions, "high risk" and personality studies in normal volunteers, studies of the effects of various types of stress, and studies of the measurement of ANS activity.

B. Methods Employed

The general methods of these studies include measurement of ANS activity through skin conductance (SC) usually measured bilaterally, heart rate (HR), vascular activity (skin temperature and finger pulse volume), and respiration while subjects are resting, exposed to a series of nonsignal tones of constant or variable intensity, and performing tasks. Tasks include tests of attention using reaction time techniques, tests of perceptual speed using two-flash discrimination and tachistoscopic recognition, and tasks designed to be moderately stressful. A mini-computer system is used to run the experiments and to collect and analyze the data. Studies in various stages of completion are listed below.

1. Schizophrenia Studies

a. A study of newly admitted, drug-free patients used three tasks varying in stressfulness and task demands to test the hypothesis, developed in previous studies, that schizophrenics' ANS does not respond appropriately to variations in stimulus significance. This study also includes several rest

periods and a series of nonsignal tones for comparative purposes.

b. In current studies, ANS recording is being carried out in two sessions which include rest periods, a tone series, and two reaction time tasks. In addition, several methods of assessing attention deficits using reaction time (RT) techniques are being compared: (1) the classical "set" procedure of Shakow which involves variations in the foreperiod in a simple auditory RT paradigm, (2) RT to visual and auditory stimuli, measured when the stimuli are predictable, unpredictable, or simultaneous (but unpredictably so) from which we can estimate attentional bias toward visual stimuli or visual dominance, (3) comparison of ipsimodal vs. crossmodal sequences of tones and lights in a simple RT paradigm, plus occasional simultaneous presentation to assess "intersensory facilitation."

c. Patients have been tested during their hospitalization using a protocol of rest periods, a series of variable intensity tones (60-100 dB), and a two-flash discrimination procedure. Patients in this study are on an active treatment or placebo. Drugs, such as pimozide, lithium, naloxone, GHB, verapamil, propranolol, and prazosin, and treatments such as hemodialysis and plasmapheresis, are evaluated.

2. Studies on Nonschizophrenic Psychopathology

a. Several confirmed psychophysiological "markers" of schizophrenic pathology have been detailed in previous annual reports and are summarized below. In order to determine which of these are specific to schizophrenia, patients with other types of psychopathology are being tested on the initial part of the current standard protocol (described in 1.b. above) after being medication free for an appropriate time. These include patients with major depressive, obsessive-compulsive, and panic-anxiety disorders (see Z01 MH 00071, 02184, 00153, and 00336). In addition, a group of young men who had a diagnosis of early infantile autism have been studied (see Z01 MH 00173).

b. In some groups drug effects are being evaluated. A placebo-controlled study of the comparative effects of clorgyline and clomipramine in obsessive-compulsives was detailed in earlier annual reports and has been published. Some panic-anxiety patients are tested on imipramine (double-blind) and will be compared with patients given placebo, and some we are able to test under both treatments. We are starting a controlled study of the psychophysiological and attentional effects of alprazolam in this group. This involves a challenge with yohimbine--a noradrenergic alpha-2 antagonist--using a protocol containing rest periods, a mental arithmetic stress, and a continuous performance task.

c. Some studies are assessing state changes independently of pharmacological treatment. Earlier annual reports described studies of state changes in multiple personality patients and phase effects in women who develop premenstrual affective symptoms. In collaboration with CHP we are doing follow-up studies on formerly adolescent patients with obsessive-compulsive disorder. Some of these patients should be basically free of symptoms, allowing us to separate out "state vs. trait" influences.

d. Patients with early stage Alzheimer's disorder are being tested with the aim of discerning if aspects of memory such as those involved in habituation of physiological responses and sequence effects in reaction time studies show deficits comparable to episodic memory deficits.

e. A group of patients with high levels of plasma norepinephrine (NE) are being tested on our standard protocol in collaboration with LCS and NHLBI and are compared with patients with similar diagnoses who have low NE. Currently we are sampling from a hypertensive population. These patients have been observed to have rather amorphous psychiatric symptoms and comparisons of their data with those from patients of known diagnosis may help to place them in a spectrum of psychiatric disorders. This study should also help clarify the role of NE in psychophysiological correlates of psychopathology.

3. Studies on Normals

a. Several "high risk" type studies are in various stages. Testing of college students selected as being extremely good or poor on two behavioral "markers" for schizophrenia (impairments of attention and eye tracking) have been described in previous annual reports. A more conventional genetic high risk approach is being planned. This, in collaboration with CNG, involves testing the offspring of patients with bipolar affective illness. The interest is in investigating whether some of the putative biologic trait markers for affective disorder reported in the psychophysiological literature can be considered to be genetic markers.

Another similar approach is the investigation of the psychophysiology of "psychoticism" or "schizotypy" as defined by questionnaires and has been described in Z01 MH 00491.

b. Two new studies of the simultaneous ANS and blood chemistry reactions to stress in normal volunteers have begun. In one with BPB and NSB, using the procedure described in 2.b. above for panic-anxiety patients, the effects of yohimbine or placebo on baseline level and reactions to two psychological stressors are being compared. This should help evaluate the role of the NE neurotransmission system in ANS activity.

Another study, with NSB, is designed to compare temporary states of "learned helplessness" and active coping on learning, mood, ANS activity, and plasma catecholamines and cortisol. Subjects are given the task of learning how to turn off an aversive noise. In one condition the problem is insoluble and, in the other, it can be solved by correctly pushing a button. The object is to produce a temporary model depressed state in humans for possible use as a tool in pharmacological and other studies.

c. A study on 95 normal subjects tested the hypothesis that ANS activity mediates the relationship between platelet MAO activity and the personality trait of sensation seeking, a relationship that has been replicated. This study in collaboration with LSES (see Z01 MH 00674) also uses a method of confirmatory factory analysis to reduce psychophysiological data as described

in previous annual reports.

4. Literature Review

A literature review of the psychophysiology of psychopathology was brought up to date during the year and is in the final prepublication stage.

C. Major Findings

1. Schizophrenic Studies

a. In previous work on schizophrenia we have shown that, in general, these patients, when unmedicated, have high levels of some, but not all, indices of ANS arousal at rest, reduced phasic and tonic reactivity, particularly to stimuli with signal value and in situations that are stressful and/or involve performance of tasks, and show slow rates of adaptation and habituation. Questions have been raised about the extent to which this ANS pattern may be involved in the pervasive deficits in attention and information processing in these patients. Specifically, an "inverted-U" model has been proposed in which behavioral deficits are attributed to excessively high or low levels of ANS arousal. The data from our completed study on a hetrogeneous sample of schizophrenics confirm our previous results on just acutely ill patients at quite highly significant levels. However, little support for the inverted-U hypothesis was forthcoming. Since the patients had high base levels and small increments of ANS activity to initiating task performance, their levels during the first part of the tasks themselves were about the same as controls. During one task and to some stimuli, patients declined in ANS activity at a slower rate than controls but in some cases the groups did not differ. Thus, differences in ANS base levels and reactivity depended on the type of task, type of stimulus, and time within a task. Behavior deficits are usually assumed to be relatively constant. If so, then there is not a simple or consistent relation between task performance and ANS activity. Further analyses which take into account individual differences in performance and ANS activity are being planned to test other versions of the inverted-U hypothesis.

b. Data are still being collected. The new data continue to show that the phenomena of visual sensory dominance and intersensory facilitation found in normal subjects also occurs in schizophrenics.

2. Studies on nonschizophrenic psychopathology

a. Preliminary findings on unmedicated obsessive-compulsive patients reported last year are standing up with further analyses. Adults show higher baseline arousal than controls but no differences in habituation. In the adolescent study, only the boys showed this pattern. Statistically, comparisons with controls showed that obsessive boys tended to be higher in ANS activity than control boys while obsessive girls tended to be lower than control girls. Obsessive boys also showed a significant deficit in two-flash discrimination performance while in girls there was a trend in the opposite direction. These results remain puzzling. Data for other groups are still

being collected or being analyzed.

b. The drug study on adult obsessives described last year has been published. Other studies are still in progress.

c. The multiple personality project results were reported last year. The premenstrual study has been discontinued due to logistic difficulties of bringing outpatient women into the laboratory when they were definitely symptomatic.

d. & e. No reportable findings as yet as we are still testing patients and/or controls.

3. Studies on Normals

a. The data from these studies have either been described in previous annual reports or are being collected.

b. These studies are in progress.

c. This study has been submitted for publication. In their final form the results show that the confirmatory factor analysis technique produced models of physiological activity that were quite similar for men and for women and across different situations. This enabled us to simplify the presentation of the substantive results. The hypothesis that the MAO sensation-seeking relationship was mediated by ANS activity was not confirmed. ANS activity was negatively correlated with sensation-seeking and positively correlated with MAO activity primarily in women, while sensation-seeking and MAO activity were negatively correlated to about the same degree in men and women. In addition, even for women, the specific ANS factors showing significant correlations were different for sensation-seeking and MAO activity. Men, in contrast, showed positive correlations between ANS activity and an active life style. The sex differences and response specificity in these relationships make it unlikely that ANS activity mediates the relationship between sensation-seeking and MAO activity.

Significance to Biomedical Research and the Program of the Institute

Investigations of ANS activity and attention in psychiatric disorders, especially schizophrenia, have produced promising results which suggest that these processes may play fundamental roles in the etiology and expression of the disorders. Limitations on inferences to be drawn from measures of ANS activity come from incomplete understanding of their biological and psychological determinants. One of the main goals of this research is to increase this understanding by investigations of biological and psychological correlates and improving measurement techniques. The dynamic nature of these measures permits the study of processes, such as adaptation, habituation, response to and recovery from stress, and effects of single stimuli through noninvasive techniques. Thus, further understanding of their mechanisms could greatly increase their utility in investigations of psychopathology. Continued investigations of the diagnostic specificity of these processes and

of their relationships to other clinical features and to prognosis are necessary to confirm and extend our previous results and to test the limits of their generality.

Proposed Course

Analysis of data will continue for the completed project on schizophrenia with the goals of determining the relationship of ANS variables to diagnosis, diagnostic subtype, symptomatology, severity of psychosis, performance on tests of attention and perceptual speed, degree of improvement during hospitalization and improvement on specific treatments. ANS activity in patient and control groups will be studied in relation to data obtained from biochemical assays of body fluids such as monoamines and their metabolites in CSF and monoamine oxidase activity.

Collection and analysis of data will continue for current projects on schizophrenic and nonschizophrenic psychopathology and in normal controls. This protocol will be used in the collaborative LPP project on attention disorders. Concept modeling by confirmatory factor analysis may be extended to other aspects of the large sample of controls and tried on data from schizophrenic patients. Investigation of ANS and behavioral effects of various pharmacological therapeutic agents will continue for all these groups with the purposes of determining the comparative effects of the drugs and correlates with clinical response.

We are planning to design a new protocol for schizophrenics by which to test the hypothesis that schizophrenics and controls exhibit differential effects of increases in arousal on attention. This will require choosing tasks that vary in their sensitivity to arousal manipulations. Arousal will be manipulated by changes in posture. Our experience with this technique in normal voluteers shows it to be effective in altering peripheral indicators of arousal without concomitant distraction.

Publications

Zahn, T.P. Psychophysiological approaches to psychopathology. In Donchin, E., Porges, S.W., and Coles, M.G.H. (Eds.). Handbook of Psychophysiology, New York: Guilford Press, In Press.

Zahn, T.P., Insel, T.R., and Murphy, D.L. Psychophysiologic changes during pharmacologic treatment of patients with obsessive compulsive disorder. British Journal of Psychiatry, 145:39-44, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00486-13 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychophysiological Effects of Stimulant Drugs in Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Theodore P. Zahn, Ph.D.	Research Psychologist	LPP, DIRP, NIMH
Other:	Judith Rapoport, M.D.	Chief	CHP, NIMH
	Martine Flament, M.D.	Guest Researcher	CHP, NIMH
	Marcus Kruesi, M.D.	Clinical Associate	CHP, NIMH

COOPERATING UNITS *(if any)*

Child Psychiatry Branch, NIMH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH/ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tests of the effects of acute and chronic administration of caffeine on autonomic nervous system (ANS) functioning have been carried out to evaluate the role of ANS activity in behavioral and subjective effects of this drug. A test of attention using a reaction time method is included.

The test protocol involves recording peripheral indicators of ANS activity such as skin conductance (SC), heart rate (HR), and skin temperature during a session consisting of a rest period, presentation of a series of simple tones to which no response is required, and the reaction time task. Studies have been carried out on the effects of the acute administration of two doses of caffeine and a placebo in 6-13 year old boys and in men, and a pilot study and major study of chronic (2 week) caffeine intake in children.

The effects of both acute and chronic administration of caffeine were increases in SC indices of arousal but some trends toward decreases in HR. The SC results are consistent with the hypothesis that caffeine can be considered a pharmacologic model for anxiety, but the HR effects suggest the model is imperfect.

In a current study, an acute dose protocol with caffeine will be done on children with anxiety disorders and on offspring of adults with anxiety disorders. This will test the hypothesis, for which there is evidence in adults, that patients with anxiety disorders are more sensitive to caffeine than controls. In addition, the study tests the hypothesis that the hypersensitivity is under genetic influence.

Project Description

This project has evolved from the study of hyperactivity in children (now called Attention Deficit Disorder) to the study of stimulant drugs--dextroamphetamine and caffeine--in normal children and adults.

Results of the caffeine studies with normal subjects have been presented in previous annual reports. The acute dosage studies have been written up and submitted for publication. The pattern of ANS results for caffeine was different from that of another "stimulant drug"--dextroamphetamine--in that caffeine produced very consistent and strong increases on SC activity but minimal or opposite effects in HR, while amphetamine dramatically increased HR and had less consistent effects on SC activity (although it also generally increased it). This is no doubt due to the different neurotransmitters involved and suggests that SC activity is more under control of the adenosine (or possibly benzodiazepine) system while HR is more controlled by norepinephrine.

The current study on anxiety disorders, mentioned above, uses the same protocol as in some of our acute studies. Children are tested at baseline, then in three sessions where they receive, randomly, 0, 3, and 10 mg/kg of caffeine one hour before testing. Three groups of children will be tested: those with a diagnosis of anxiety disorder, those with a first-degree relative (parent, sibling) who has an anxiety disorder but who are free of psychopathology themselves, and normal controls.

Significance to Biomedical Research and the Program of the Institute

The ANS effects of caffeine consistently found in these studies partially resemble those seen in anxiety states and other psychopathology. Caffeine has been shown to affect adenosine and benzodiazepine receptors. Thus, it appears that ANS activity is partially controlled by these systems and may help mediate their effects on the symptoms of anxiety. These studies also suggest that dietary choices may be influenced by ANS activity. The demonstration of anxiety-like physiological effects of caffeine suggests that some persons may not tolerate it well. This would seem to have implications for the controversy over product labeling. Since dextroamphetamine is clinically beneficial to hyperactive children and caffeine is not, comparison of their physiological effects on the same protocol may give us clues about the mechanisms responsible for amphetamine's therapeutic effect.

Proposed Course

Planned future research includes psychophysiological study of children with attention deficit and conduct disorders in connection with CSF measurements of biogenic amines and their metabolites. There is evidence linking serotonin levels, SC activity, and certain behavior disorders with each other. We also plan to include a detailed examination of the nature of the attention deficit in attention deficit disorder in line with the general program of this laboratory to develop a taxonomy of attention disorders.

Publications

(See #Z01 MH 00161-07 CHP)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00491-09 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Personality Factors and Psychophysiological Responses to Changing Stimulus Input

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Theodore P. Zahn, Ph.D.	Research Psychologist	LPP, NIMH
Other:	Thomas N. Robinson, Jr.	Guest Researcher	LPP, NIMH

COOPERATING UNITS *(if any)*

NIH Normal Volunteer Office.

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NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.5	0.0

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to investigate relationships among differences in personality, sensory thresholds, and autonomic nervous system (ANS) activity in normal humans and to study racial differences in ANS activity. Bilateral skin conductance and heart rate have been recorded in two sessions in which constant and variable intensity tones and lights are presented and auditory and two-flash thresholds (TFT) determined by methods which permit signal detection analyses. Several standardized personality tests were also given. These include scales of sensation-seeking, extraversion, neuroticism, psychoticism, field dependence and anxiety. In addition comprehensive measures of lateral dominance have been given as well as a measure of "torque" (clockwise drawing of circles) which is thought to reflect a neurointegrative deficit and be related to risk for future psychopathology. The procedures allow determination of the effects of stimulus intensity and heteromodal stimulation on ANS activity. A procedure for manipulating ANS arousal experimentally with minimal distracting effects--a change in posture from supine to standing--is being used to assess the effects of arousal on performance and the effects of personality variables on this relationship. This project allows testing of several theoretical models of the relationships of ANS activity, sensory sensitivity, and personality, some of which have implications for the etiology of psychopathology. Tests of the relationships between laterality in skin conductance variables and behavioral laterality will also be done to see if inferences about lateralized brain function can be made from such variables.

Project Description

A. Objectives

A large body of psychological literature postulates that an important dimension of individual differences in behavior or personality is reflected in the reactions of the nervous system to sensory stimulation. Pavlov's original conception of "strong" and "weak" nervous types has been modified and extended by Western theorists to reflect such personality dimensions as "extraversion-introversion," "sensation-seeking," and "field dependence," each of which can be measured by a questionnaire or other test procedures. The theoretical models that have been built up from these concepts have implications for interrelationships among personality, autonomic nervous system (ANS) base levels and responsivity to stimulation, and sensory sensitivity. There are also implications for psychopathology, in that schizophrenics have been considered to be extremely "weak" nervous types in the Pavlovian system (i.e., overreactive to weak stimulation and underreactive to strong stimulation--"transmarginal inhibition"). Another development is the more recent delineation by H. Eysenck of the dimension of "psychoticism."

The major objective of this project is to test some of the implications of these models of personality by interrelating the personality measures with sensory thresholds and sensitivity, and ANS activity in normal humans. Other objectives are to assess racial differences in ANS activity and in its relationships to the other variables in the study and to explore relationships of differences in the laterality of skin conductance activity with behavioral assessments of laterality, and to test the effects on ANS activity increasing arousal by means of a postural change.

B. Methods Employed

Over 200 normal volunteers have been assessed on several personality dimensions, including the Eysenck scale of extraversion, neuroticism, and psychoticism, field dependence, sensation-seeking, impulsivity, ego strength, and anxiety, assessed for degree of lateral dominance, and given tests of ANS and sensory functioning in two separate sessions as described earlier.

In a second protocol, the effects of changes in posture on ANS activity during rest, a series of 86dB tones and a TFT task is assessed. Subjects are tested when they are reclining or standing on two separate days in counterbalanced order.

C. Major Findings

In previous annual reports, relationships between questionnaire-defined personality variables, ANS activity, and sensory thresholds have been described. In general, subjects with high scores on the Eysenck personality scales of extraversion, psychoticism, and, surprisingly, neuroticism tend to have low ANS activity and reactivity. Subjects high on sensation-seeking were also very responsive autonomically to novel stimuli. Low sensory sensivity was shown by subjects high on psychoticism and those showing a "torque"

(clockwise) pattern of drawing a circle.

In a recent analysis of the data, subjects' electrodermal responsivity to a series of nonsignal tones was taken as the independent variable. This analysis was undertaken because these "orienting responses" have been reported to be deviant in many types of psychopathology. Nonresponsiveness or hyporesponsiveness has been found in some schizophrenics and endogenous depressives while a failure of habituation has been observed in some schizophrenics, agitated depressives, and patients with anxiety disorders. Four groups were discriminated according to the number of skin conductance orienting responses (SCOR) they gave to a series of 8 73 dB tones: Nonresponders (NR, 0 SCOR) fast habituators (FH, 1-2 SCOR), normal habituators (NH, 3-6 SCOR), and slow habituators (SH, 7-8 SCOR). As expected these groups differed in ANS reactivity in the expected direction in other parts of the session, but mainly in electrodermal variables. However, in heart rate (HR) responses in the SH group tended to show an accelerative HR response (usually associated with "defensive" responding) even to moderate intensity tones, while the other groups showed the more common decelerative "orienting" reaction.

On the perceptual tasks the NR group had a higher two flash threshold and lower auditory sensitivity (d') than the more reactive groups. Thus, these autonomically hyporesponsive normal subjects were less sensitive to environmental stimuli. This result confirms the Pavlovian idea that orienting responses serve to heighten the sensitivity of "sensory analyzers". The relationships between the responder categories and the personality measures were generally small and nonsignificant.

Significance to Biomedical Research and the Program of the Institute

Further understanding of how autonomic, perceptual, and personality variables interact in normal subjects should be of great assistance in interpreting the autonomic and perceptual results from studies on psychopathology in which similar methods are used in our other studies. Similarly, the study of racial differences in normals will help us evaluate the results of racially mixed samples of patients. This project has been very useful in the development of protocols for studies of psychopathology.

Proposed Course

Much further analysis of this large data base remains to be done, including assessment of laterality effects and determination of the effects of other personality dimensions in the most recent protocol. Techniques such as confirmatory factor analysis might be useful in such analyses.

We are planning also to develop a new battery of tests that vary in their sensitivity to arousal, making use of some of the recent developments in the field of cognitive psychology.

Publications

Robinson, T.N., Jr. and Zahn, T.P.: Psychoticism and arousal: Possible evidence for a linkage of P and psychopathy. Personality and Individual Differences. 6:47-66, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00495-09 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Psychobiology of Cognitive Processes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Herbert Weingartner, Ph.D. Chief, Unit on Cognitive Studies LPP/NIMH

COOPERATING UNITS *(if any)* Biological Psychiatry Branch, Laboratory of Clinical Science, Clinical Neuropharmacology Branch, Clinical Psychobiology Branch, NIMH; NIAAA; NIDA; Laboratory of Neurosciences, NIA (NIH); NINCDS (NIH); Walter Reed Hospital

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of these research efforts is to explore the psychobiology of cognition in man. Studies are carried out that would help define the psychobiological determinants of components of cognition that are necessary for learning, memory, and related mental functions. Studies have explored the specific and discrete mechanisms which account for the acquisition, processing, encoding, consolidation, and retrieval of experience. Clinical studies are developed programatically to further our understanding of the biological and psychological determinants of impaired cognition in psychiatric and neuropsychiatric patients. Understanding the central nervous system effects of drugs of abuse in terms of their cognitive expression represents another important research direction that is presently underway. Aspects of state-dependent learning and retrieval of the discriminative properties of drugs, affect and cognition and the role of the reward system in cognition are all presently being studied. Specific forms of central nervous system dysfunctions (e.g., as defined by type of lesion in neuropsychiatric disorders) may affect distinct components of cognitive processing. Similarly, psychoactive drugs that affect discrete aggregates of neurons, may alter different aspects of cognition and information processing and serve to model forms of cognitive dysfunctions in man. Based on empirical studies of clinical populations (e.g., Depression, Alzheimer's disease, Huntington's disease, Korsakoff's disease, forms of learning impairments in children) and several types of psychoactive agents (cholinergic drugs, noradrenergic drugs, serotonergic drugs, drugs that alter GABA activity, neuropeptides), it has been possible to begin to define the psychobiological relationships between knowledge and episodic memory, encoding processes, effortful (active) cognitive operations as opposed to automatic cognitive processes and how retrieval processes are mediated by the context (state of central nervous activity).

Other Professional Personnel

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Project Description

The research projects reviewed here are all concerned with the psychobiology of cognitive processes. They have been designed to explore the psychological and biological determinants of various aspects of cognitive processes and their interrelationships. Studies reviewed here have examined and contrasted various types of cognitive disorders associated with psychiatric and neuropsychiatric diseases. Parallel research has examined how specific neurochemical systems mediate different types of cognitive processes. The types of processes that have been examined including the encoding, processing, learning, and storage of information, how processed events are altered, or elaborated in memory, the consolidation and retention of information, and the mechanisms that are involved in retrieval of stored information. Other research efforts have also begun to examine how we know what is and is not in memory (meta-cognitive processes), attentional determinants of information storage, aspects of short-term memory, the consolidation of information, long-term memory, state and trait specific cognitive strategies, effort demanding vs. automatic cognitive processes, and the kinds of strategies that subjects use to retrieve previously acquired knowledge and skills. Recent studies have also focused on the distinction

between the psychological and biological determinants of episodic and semantic (knowledge) memory, meta-cognitive processes associated with information processing, the role of the reward--reinforcement system for memory processes, how affect mediates cognition, and the cognitive reflection of the discriminative properties of drugs.

A. Current Research Strategies

Two types of approaches or strategies are used to explore the psychobiology of cognitive processes. One is to contrast the effects of different treatments on different components of cognition. These studies include: (1) pharmacological manipulations, such as serotonergic drugs, drugs that alter GABA, cholinergic drugs, noradrenergic drugs, abused drugs (alcohol, marijuana), central nervous system depressants, neuropeptides, and (2) behavioral manipulations that alter reinforcing properties of stimuli, arousal/activation, stimulus attributes (altering encodability), and types of stimulus processing strategies subjects use to process information. These studies are carried out in unimpaired subjects, as well as in patient groups with different forms of psychopathology such as disorders of mood, dementing disorders and in patients with localized brain injuries. Would different kinds of pharmacological or behavioral manipulations of cognition lead to different forms of enhanced or disrupted cognition? Contrasting different treatment effects on different CNS systems and relating these changes to cognitive responses should be particularly useful in providing us with a picture of the structure of the psychobiology of cognition.

A second type of strategy for researching the psychobiology of cognition is to contrast different forms of cognitive failures as seen in different psychiatric and neurological syndromes. Would disruptions in cognition seen in some psychopathological states be qualitatively and quantitatively unique and related to specific changes in central nervous system activity or the neuropathology of these disorders? For example, what are the differences in the amnestic and cognitive impairments seen in Huntington's disease, Korsakoff's syndrome, and Alzheimer's disorder? To what extent are cognitive changes associated with aging or the "pseudodementia" evident in some Parkinson's disease patients different from impairments evident in dementing disorders? How might the differences be an expression of the specificity of central nervous system involvement in each of these disorders? In some instances, the possibility of discriminating between the form of the cognitive impairment is necessary for both adequate diagnosis and effective treatment, i.e., such as the cognitive disruptions that are part of depression as opposed to that seen in a progressive dementia of an Alzheimer's type. Frequently, depression is an integral part of a progressive dementia, and the cognitive impairment is a joint product of the two disorders. Some studies have also investigated the therapeutic potential of various psychoactive drugs and behavioral treatments. Do such treatments attenuate or reverse the cognitive disruptions seen in various forms of dementia, hyperactivity, amnesias, and learning disability syndromes in children, depression, and the schizophrenias?

In summary, each of the studies is clinically relevant but also serves as a basis for understanding the underlying psychobiological determinants of

cognitive processes. Each project described below is concerned in some way with defining the discrete psychobiological components of cognitive processes that are involved in the appreciation, storage, retention, and retrieval of experience and using that knowledge to understand and treat disordered cognition.

B. Methods of Approach.

The general methods of approach used to study the psychobiology of cognition are detailed in the last annual report. In all of the studies currently underway different processes are systematically contrasted to one another in order to define islands of impaired and spared cognitive functioning. This is done in studies in which different clinical populations are compared to one another and in parallel research where different cognitive processes or domains are contrasted in terms of specifiable alterations in general aspects of cognitive functioning. This broad approach has been developed in a series of review-theory papers. In design of any single study three strategies have been used. One involves manipulation of different biological systems that may play a role in different aspects of cognition in man. Various neurotransmitter agonists and antagonists, as well as agents that affect neuroendocrine functioning are contrasted in both impaired and unimpaired subjects. A second strategy involves systematic comparison of various forms of cognitive failures apparent in different clinical groups. Methods used include measures and assays for evaluating neuropathological, neurochemical, and neuroanatomical changes that are apparent in different clinical syndromes. These data provide a matrix for relating biological variables with measures of different components of cognition as seen in forms of impaired cognition. The third set of methods involves systematic manipulation of acquisition conditions, stimuli, retention processing, and retrieval conditions. The specific methods of approach involve seven types of study designs. These include:

a. Semantic (Knowledge) Memory and its Relationship to Other Forms of Learning and Memory (Episodic Memory);

b. Pharmacological Alterations (Enhancement and Disruption) of Cognitive Processes; Changes in Mood, Enhancement and Disruption of Higher Mental Functions;

c. State-Dependent Learning - State Dependent Retrieval (Context Dependent Memory);

d. Memory Consolidation;

e. Behaviorally-Defined Mechanisms that Alter Components of Cognition;

f. Cognition and Mood;

g. Strategies for Scaling Cognitive Impairments.

C. Findings: Psychobiology of Cognition

1. Neuropharmacological Studies of Cognition in Man

In a series of studies it has been possible to neuropharmacologically model different types of cognitive dysfunctions associated with distinct neuropsychiatric disorders. The benzodiazipines produce dose dependent impairments in memory that are structurally very like that seen in non-dementing types of amnesia. Episodic memory functions are disrupted while access to previously acquired knowledge remains intact across a wide dose range. This effect is consistent across a number of benzodiazepines. The effects of benzodiazipines on memory are intimately linked to the sedative properties of these drugs. Anti-cholinergic drugs produce a strickingly different type of cognitive impairment in unimpaired subjects. These drugs, particularly scopolamine, produces a cognitive response mimicking SDAT. Memory effects are not linked to sedation. Access to knowledge memory is disrupted in a dose dependent fashion. Inability to access and use previously acquired knowledge determines other features of the cognitive failure following scopolamine treatment. It has also been shown that this type of cognitive dysfunction is similarly evident in SDAT patients. However, these patients are particularly sensitive to the effects of cholinergic antagonists. This type of neuropharmacological challenge is currently being used to characterize and perhaps diagnose patients with cognitive symptoms resembling SDAT.

There appears to also be specificity and distinctiveness in the role of neuropeptides such as synthetic vasopressin-like substances, and of naloxone, in determining aspects of learning and memory in both cognitively impaired patients (depressed patients, alcoholic Korsakoff amnestic syndrome patients and progressive dementia patients) as well as in unimpaired subjects. Different neurotransmitter systems and different kinds of neurochemical mediators are involved in the regulation of various aspects of episodic memory (acquisition, retention, and retrieval of information) while other biological determinants appear to influence semantic memory pro- cesses. Furthermore, effortful cognitive operations appear to be determined by different biological mechanisms from those involved in automatic cognitive operations.

Based on a series of studies using various types of drug strategies it has been possible to demonstrate a double dissociation between automatic cognitive operations and those that require effort considerable cognitive capacity. These findings provide further important evidence pointing to the psychobiological distinctiveness of different aspects of cognition.

2. Cognitive Impairments in Neuropsychiatric disorders

Memory-cognitive dysfunctions in different neuropsychiatric disorders are determined by different mechanism. Findings contrasting these different forms of cognitive dysfunction are important for appropriate diagnosis, potential treatment and has also served to provide information about the biological bases of learning and memory. In the progressive dementias of the Alzheimer's type information is lost rapidly from memory; immediate memory is often relatively unimpaired, and any type of learning-memory operation that requires the establishment of permanent trace events in memory is dramatically

disrupted. Memory failures are, in large part, due to processing or acquisition deficits which then result in weak trace formation and therefore failures to retain information in memory. A considerable body of research has suggested a distinction between semantic memory and the repository of information of knowledge structures from episodic memory, i.e., memory for ongoing recent events. Although these two kinds of memory systems have been traditionally viewed as being separate and distinct, we have found an important link between the two. Based on recent findings relating these two systems, it has been possible to account for many aspects of the memory impairment in progressive dementia patients. It has been possible to demonstrate that Alzheimer's patients have difficulty accessing structures in semantic memory. This impairment is directly linked to the severity of their recent, episodic memory impairment. These results have important implications both diagnostically, in distinguishing this group of cognitively impaired patients from other groups (e.g., cognitively impaired depressed patients), as well as for the development of potential treatment strategies.

Parkinson's Disease (PD) patients also demonstrate learning-memory problems that can be quite severe. We have found these impairments to be qualitatively different from those evident in Alzheimer's disease. PD patients manifest impaired cognition on effort demanding cognitive tasks but not when information can be processed relatively automatically. Access to semantic memory is also left unaffected in the early and middle stages of the disorder. L-dopa treatment, a common drug used in PD, produces a facilitation of these same cognitive component processes in unimpaired older subjects.

A number of clinical studies are continuing with the aim of facilitating learning and memory in progressive dementia patients. Two very different strategies are being employed. Cholinergic agonists seem to produce small improvements in learning and memory but only in those patients that are least cognitively impaired. In contrast, arginine vasopressin enhances learning and memory by facilitating access to semantic memory (a mechanism of action that is consistent with the determinants of the memory failure in these patients).

Although Korsakoff patients (KD) are often as memory impaired as progressive dementia patients, the cognitive and biological determinants of their impairments are quite different. Unlike progressive dementia patients, the Korsakoff amnesia patient responds to attributes of stimuli that would ordinarily aid encoding such as a) repeating information, b) organizing information, and c) presenting pictures rather than words. Furthermore, the Korsakoff patient (KD) can learn procedures and remember them for very long periods of time. This is because unlike progressive dementia patients the Korsakoff patient (KD) is able to access semantic memory.

In attempting to reverse the amnestic-like impairment in KD we have tried drugs that would affect the noradrenergic system. Thus far we have been unsuccessful in producing reliable improvements in KD memory functions using clonidine as a drug strategy.

These findings, when examined together, have suggested that cognitive failures in progressive dementia are distinguishable from those evident in

depression and other syndromes. This has prompted active study of drug and other treatment strategies for reversing such cognitive failures. By understanding both the mechanisms of cognitive impairments and the neurochemical response following various forms of drug treatment, it should be possible to design studies that would examine the therapeutic potential of various types of drug treatments. The mechanisms and determinants of the cognitive impairments in depression and dementia have allowed us to devise strategies that should prove useful in distinguishing between these two groups of patients. Characteristics of automatic versus effortful processing, the extent to which effort is extended in accomplishing tasks, and the processing of unrelated vs. related events allows us to begin to differentially diagnose the cognitive impairment in depression from that seen in the progressive idiopathic dementia patients.

Newly developed methods will allow us to identify cognitively impaired patients who are more likely to benefit from different types of drug treatment. Other recent research has provided new approaches to behavioral rehabilatory techniques as well as approaches to longer term drug treatment in cognitively impaired patients.

3. Cognitive Changes in Depression

The pattern and determinants of cognitive changes in depression have been shown to be distinguishable from those expressed in other disorders (particularly in early stage progressive dementia). Depressed patients demonstrate a type of disordered thinking, one that is manifest in an inability to accomplish focused, sustained analysis of information leading to impairments in concept learning, acquisition of information, and memory. This may be related to alterations in the function of cerebral lateralization involved in processing language vs. non- language information.

A series of studies have been designed to examine the effects of focal, CT scan defined, lateralized brain lesions on mood (depression) and cognition. As expected, non-dominant hemisphere lesions produce cognitive dysfunctions that are systematically different from those produced by dominant hemisphere lesions. Non-dominant hemisphere lesions of the temporal-parietal region are also associated with profound states of depression that are obvious to trained observers but are not experienced, as such, subjectively. In contrast, patients with dominant hemisphere lesions experience depression that is consistent with observer (clinically evaluated) rated depression: in addition, the severity of cognitive dysfunction is highly correlated with the severity of depression in dominant hemisphere patients.

4. Learning Disabilities in Children

Drug treatments, such as stimulants, appear to facilitate learning and memory in some types of learning disabled children. These cognitive effects are seen primarily for those processes that require sustained effort. These effects are apparent and independent of other clinical changes in these amphetamine-treated children. In addition, learning that occurs in the amphetamine-treated state does not appear dissociated when remembering takes

place in the untreated state. This is not like the kinds of dissociative, state-dependent, learning and memory effects that are seen in stimulant treated adults. These results are also important in considering the effects of stimulant treatment on the educational experience of learning disabled or hyperactive children.

In a series of studies, we have attempted to describe the components of cognitive changes that are apparent in children with various forms of learning disability. We have examined two groups of these children, one where hyperactivity is part of the syndrome, and a second group where there is no evidence of hyperactivity or generalized retardation. Nevertheless, these children demonstrate dramatic impairments in learning and memory that resemble the kinds of disruptions in cognition that are evident in some groups of adults. The resemblance is closest to depressed patients; it also resembles the kinds of cognitive changes that are produced by drugs that disrupt cholinergic and norad-renergic activity. These children show impairments in effortful processing of information; automatic processing is left relatively intact. On incidental learning paradigms, these children are indistinguishable from normal controls. Both groups of children also demonstrate impairments in those characteristics of cognition that require the imposition of organization in memory. In many ways, the results we have obtained to date would suggest that the type of cognitive impairment seen in these children resembles that seen in depressed patients in contrast to the pattern of cognitive impairments evident in progressive dementia patients. The kinds of cognitive impairments are also like those that are apparent when unimpaired subjects are treated with drugs that disrupt or block catecholamine activity.

5. Defining the psychobiology of distinct cognitive processes

Studies of patients with lesions in various parts of the brain, defined by CAT scan findings, has demonstrated that several types of cognitive and non-cognitive processes are psychobiologically distinct, but interactive systems. For example, in a study of 550 Vietnam veterans who sustained head injury 10 to 15 years ago, it has been possible to demonstrate that lesions of the dominant vs non dominant (with respect to language)hemispheres produces different types of disturbances in mood and related changes in cognition. Some lesions previously thought to produce a dementia like picture in SDAT patients did not produce this type of dementing picture in those patients. The most striking finding from the study of those patients has been that pre-injury intelligence more so than either amount or location of brain injury accounts for the maintenance of effective intellectual functioning years after the brain injury. These findings have important implications for our understanding of brain plasticity.

Studies have shown that semantic memory (knowledge memory) and episodic memory are psychobiologically distinct but interactive cognitive systems. Both drug studies in unimpaired subjects and comparative studies of memory impaired neurological disorder patients has shown a) failure to access semantic or knowledge prostructures in memory is the major determinant of the cognitive failures in many progressive dementia patients of an Alzheimer's

type (SDAT); b) that episodic memory failures are determined by semantic memory impairments in this type of progressive dementia; c) other amnestic syndromes (such as in Korsakoff's disease) are determined through different psychobiological mechanisms, other than those that affect access to semantic memory; and d) the cognitive disturbance in depression is due to a specific disruption in effort demanding cognitive operations while automatic processes and semantic memory functions are left relatively unaffected - this same pattern of cognitive impairment is also evident in early stage Parkinson's disease. These findings have been useful not only because they elucidate basic mechanisms of learning, memory and related cognitive cesses but they provide new and more effective diagnostic tools for distinguishing types of cognitive dysfunctions.

Related neuropharmacological findings include: a) neuropeptide treatments such as arginine vasopressin facilitate access to semantic memory; b) serotonergic drugs, such as Zimelidine appear to enhance memory by amplifying weak, poorly processed memory traces; c) L-dopa treatment appears to produce a specific enhancement of effort-demanding cognitive operations implicating the dopamine system in this type of memory-learning function.

Behavioral manipulations that induce priming of knowledge in long-term memory has a direct and positive effect on episodic memory performance. This new finding is also being exploited in the development of pharmacological strategies that would aid memory-impaired patients.

Summary

The past year has provided us with the bases for the development of a psychobiological system for analyzing cognitive processes in humans. It has been possible to begin to highlight relationships between different cognitive operations and to break apart aspects of memory functioning into separate components. This system has also allowed us to consider the role of non-cognitive systems such as reward, and motivation and how these are reflected in alterations in cognition. Methods for scaling types of cognitive enhancements and dysfunctions have provided the bases for comparing many types of treatments that in previous studies have been shown to alter cognition. As such, the recently completed programmatic-research efforts of the Unit on Cognitive Studies has extended our knowledge of the psychobiological structure and determinants of cognition. Completed research has been valuable in better defining disordered mood, the nature of the information processing impairments in various neurological and neuropsychiatric disorders and in response to drugs of abuse. Neuropharmacological studies in both unimpaired subjects and patient groups have provided us with detailed information about the neurochemical events important for learning, memory, and cognition. These studies have also suggested new approaches to the treatment of various forms of cognitive disturbances.

Recent studies have provided a basis for defining the conditions that must be met in clinical studies that are necessary for developing new diagnostic instruments. These findings are also used for directing research into new treatments for cognitive dysfunctions. These findings have also provided a

means for distinguishing between several types of cognitive processes. One distinction supported by recent findings is that between recent (episodic) memory and knowledge (semantic memory). The distinction between these two types of memory systems is important for our understanding forms of cognitive failure in man. We have developed a way of characterizing the nature of the cognitive changes in depression and Parkinson's Disease, and used this as a way of examining the psychobiological distinction between automatic and effortful episodic memory-learning processes. It has been possible to model forms of cognitive impairments in man such as those that are seen in Alzheimer's disease, in drug studies of unimpaired subjects (cholinergic antagonists). This type of research has helped us in our efforts to facilitate aspects of cognition in these patients using cholinergic agonists. Neuropeptides have also been used to treat some of these cognitive disorders (e.g., arginine vasopressin). We have also modeled disorders of information processing in unimpaired subjects (with the use of naloxone). Based on our recent studies that involve changes in the dopamine and serotonin system we are also able, for the first time, to differentially affect automatic vs. effortful cognitive operations and to alter recall of weak memory traces in contrast to well learned information in memory. As a result of these research efforts it seems important that we focus our new efforts in the following areas: (a) mediational processes that are involved in transforming and encoding information (using psychophysiological and neuropharmacological tools); (b) the relationship between the reward system and memory processes, particularly as these would alter memory consolidation; and (c) new ways of facilitating impaired cognitive processes.

Significance to Biomedical Research and to the Program of the Institute

These research efforts have a direct bearing on how diagnoses of cognitive dysfunction are accomplished and the directions of future efforts for treating the cognitive impairment associated with a wide variety of psychiatric and neuropsychiatric disorders.

Proposed Course

We hope that current studies will lead to better diagnostic tools and effective therapies for cognitive dysfunction.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00500-06 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cognitive and Perceptual Changes in Affective Illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward K. Silberman, M.D.	Guest Researcher	LPP/NIMH
Others:	Robert Post, M.D.	Chief	BPB/NIMH
	Jean-Phillippe Boulanger	French National Institute for Health & Medical Research	Cannes, France
	Linda Bierer, M.D.	Medical Staff Fellow	BPB/NIMH
	Thomas Uhde	Chief, AAD	BPB/NIMH
	Rex Cowdry, M.D.	Clinical Director	NIMH
	Steven Taube, M.D.	Walter Reed Army Institute of Research	

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch; Walter Reed Army Institute of Research
French National Institute for Health & Medical Research, Cannes, France

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.5	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to investigate the cognitive and perceptual changes which are present in, and characteristic of major affective illness and its various clinical and biological subtypes. Two separate studies make up the overall investigation: (1) psychomotor and psychosensory symptoms in patients with affective illness, and (2) lateralized hemispheric function in depression.

Project Description

This project is based on the idea that cognitive and perceptual changes in depression may be useful markers for sub-classification of affective disorders. At present, there are two distinct approaches to investigating such markers, which are described below.

1. Psychomotor and Psychosensory Symptoms in Affective Illness

In our initial study we have compared 44 patients with major depressive disorder to 37 patients with partial complex epilepsy and 30 hypertensive controls on the frequency with which they experience transient alterations in perception, ideation, affect, and motor behavior, similar to symptoms which have been described as seizure concomitants in the clinical epilepsy literature. In our initial study, both affective and epileptic patients reported such symptoms with significantly greater frequency than controls. Both affective and epileptic patients reported transient visual, auditory, and olfactory changes. Epileptic, but not affective patients, reported gustatory, vestibular, and tactile phenomena, as well as involuntary motor symptoms. Affective patients were distinguished by presence of cognitive illusions, and distortions of time perception. Such symptoms were experienced by affectively ill patients primarily during episodes of illness rather than interval periods. A high frequency of symptoms reported were unrelated to personality factors as measured by MMPI. With the exception of an inverse relationship to age, symptoms were not related to demographic variables, or parameters reflecting course of illness. However, those patients with better response to lithium and tricyclic antidepressants reported higher numbers of symptoms than those with poor response to these drugs.

Proposed Course

At present the focus of planned research is to investigate psychosensory symptoms in patients with other diagnoses in the affective spectrum and to study the relationship of such symptoms to clinical presentation of depressive illness, to biological parameters, and to drug response. In conjunction with Dr. Robert Post, Jean-Phillippe Boulanger, Linda Bierer, and Thomas Uhde of the Biological Psychiatry Branch, NIMH, we have investigated the occurrence of psychosensory symptoms in patients with panic-anxiety disorders, with and without concomitant depression. Plans are under way to examine the relationship of these epileptic-like symptoms to antidepressant response to carbamazepine, a drug useful in treating both affective disorders and epilepsy. To date, depressed patients both with and without panic attacks have been found to have such symptoms; the presence of both panic attacks and depression tends to produce more symptoms than in groups with only one type of disorder.

2. Lateralized Hemispheric Function in Depression

Many lines of evidence suggest that two cerebral hemispheres differ in the manner and degree to which they process emotionally-related stimuli and modulate emotional behavior. In particular, the right hemisphere has been

found to exhibit "dominance" for processing of emotions, overall, or as processing of negative emotions specifically. In affective disorder, where disturbances of mood and emotions are paramount, a variety of studies have suggested that the right hemisphere is disordered in its function, or that the balance of activity level between right and left hemispheres is shifted in favor of the right. An initial investigation from our laboratory demonstrated that, in hospitalized, depressed women, the right hemisphere appears to more efficiently process verbal stimuli which are normally handled by the left hemisphere. This finding supports the hypothesis that the right hemisphere is in some sense "hyperactive" in depressive illness.

Proposed Course

In planned follow-up studies, we will use our procedures to examine laterality in patients with a significantly depressed mood (as measured by standard clinical scales) in the context of a wide variety of disorders within the affective, schizophrenic, and personality areas. The aim of such a survey would be to replicate our original findings and to learn to what extent laterality changes are related to depressed mood itself, to diagnostic categories (e.g., affective illness, but not personality disorder with depression) or to subtypes within affective disorders. Laterality may also be an indicator of prognosis, degree of recovery, or response to medication, and such parameters will be investigated. These studies will be conducted in collaboration with Sander Genser, M.D., NIDA, and Dr. Steven Taube, Walter Reed Army Institute of Research. The patient population for the studies will be drawn from the inpatient services of the Walter Reed Army Medical Center. At present, a protocol has been prepared for submission to the Clinical Investigation Committee of the Walter Reed Army Medical Center. Space on the Walter Reed campus has been allocated to the project and we are in process of gathering and setting-up the necessary equipment, which will be supplied by the Walter Reed Army Institute of Research.

Significance to Biomedical Research and to the Program of the Institute

These investigations are part of a basic research at NIMH aimed at elucidating the nature of affective illness. Cognitively related studies are relevant to this goal from three points of view: (1) they concern an important area of deficit in affective illness, (2) they define an aspect of dysfunction which may provide clues to the pathologic anatomy and physiology of affective illness, and (3) they may provide useful information relating to clinically meaningful classification of affective disorders.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00503-05 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Clinical Studies of Attention Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Allan F. Mirsky Chief LPP, NIMH

COOPERATING UNITS *(if any)*

Epilepsy Branch, NINCDS; Clinical Neurosciences Branch, NINCDS; Laboratory of Clinical Sciences, NIMH; Neuropsychiatry Branch, NIMH; Boston University

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.25	.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research comprises three related areas of investigation concerned with specifying neuropsychological factors underlying clinical conditions in humans in which disturbed attention is a major symptom. A major emphasis is on (1) illuminating the nature of brain stem pathophysiology, if any, in such entities as petit mal or absence epilepsy, infantile autism, schizophrenia, and related diseases; (2) an additional major emphasis is on extending the neuro-behavioral analysis of attention loss in absence epilepsy so as to facilitate developing alternative treatment strategies for such patients. Both of these projects form part of a larger effort which is aimed at (3) developing a comprehensive and systematic taxonomy of attentional disorders in humans. This latter study will eventually comprise study of patients with cerebral lesions, seizures, dementing diseases, and metabolic illnesses of the brain.

Others:

Connie C. Duncan, Ph.D.	Senior Staff Fellow	LPP, NIMH
Walter H. Kaye, M.D.	Staff Psychiatrist	LPP, NIMH
Richard Coppola, D.Sc.	Senior Engineer Officer	LPP, NIMH
Theodore P. Zahn, Ph.D.	Research Psychologist	LPP, NIMH
Richard Nakamura, Ph.D.	Senior Staff Fellow	LPP, NIMH
Roger Porter, M.D.	Chief	EBB, NINCDS
Debbi Fein, Ph.D.	Asst. Professor of Psychiatry	Boston Univ.
Daniel R. Weinberger, M.D.	Chief, CNS	NPB, NIMH

Project Description

1. Brain Stem Mechanisms in Attention Impairment

Current approaches to the neuropsychology of attention impairment have emphasized that the system responsible for the maintenance of attention or consciousness within the brain is most likely represented at a variety of levels of the neuraxis. From an evolutionary point of view, it is clear that the capacity for sustained attentive behavior is present in many species which do not possess more than a rudimentary forebrain or telencephalon. MacLean's analysis of the R-complex within the human brain leads to the view that this "clump of ganglia," which constitutes virtually all of the reptilian brain, can support a variety of ritualistic, repetitive behaviors which could be characterized as sustained and attentive. Evolution progressed and the brain developed additional complexity and volume. Additional capacity for attentive behavior was thus overlaid on the more primitive, although in many aspects thoroughly adequate, brain stem system of the reptile. Therefore, although the system for maintenance of attentive behavior in the human (or higher primate) includes limbic and neocortical components, the brain stem remains a key component and possibly the keystone of the entire system. Authors such as Hughlings Jackson and Penfield and Jasper recognized this in their conceptions, respectively, of "highest level seizures" and the "centrencephalon." In their theorizing, consciousness was either localized in or regulated by deep brain stem structures. Without reviewing all of the evidence that led to those views of the hierarchical organization of attention and consciousness within the brain, we nevertheless point to the extremely deleterious effects on such capacities of small lesions in the brain stem region of the third and fourth ventricles. In the last ten years, a new technological refinement of evoked potential methodology has made possible an other-than-theoretical exploration of the role of brain stem structures in certain clinical states. This "far field" or BAER (for brainstem auditory evoked responses) technique makes it possible to assess the integrity of auditory (and somatosensory) relay nuclei within the brain stem of humans. Although the technique has probably had most utilization in the diagnosis of demyelinating disease, it has also been used in the study of other neurological and, recently, psychiatric disorders. There may or may not be any specific interest in these sensory systems (auditory, somatosensory) in studying a particular clinical entity (i.e., absence seizures, infantile autism); nevertheless, the possibility of evaluating the functional integrity of certain systems within the brain stem is extraordinarily valuable, and many

clinical investigators are using these techniques. We have published work indicating that there are disturbances (prolonged transmission time) in the processing of auditory information in the brain stem in infantile autism. We have also shown that in absence seizures (spike-wave activity), both naturally-occurring and experimentally-induced, there may be perturbations of auditory brain stem functioning. We have run approximately 13 autistic children and a smaller number of controls on brain stem evoked potentials. We continue to perform analyses on the data gathered in this investigation and hope to have some preliminary findings ready for presentation at an early date.

2. Neurobehavioral Studies in Absence Epilepsy

We have for a number of years been studying the absence attack in patients with petit mal/centrencephalic/absence seizures (the terms are more or less interchangeable) as a model state to understand the phenomenon of consciousness/attention. Some of these studies have involved comparing the behavioral capacities of patients suffering from petit mal--as opposed to focal seizure disorders; other studies have involved detailed comparison and contrast between the behavioral and the electroencephalographic symptoms/signs of the disorder. Most recently these investigations have: (1) used evoked potentials in the visual and auditory modalities as indices of the sensory effects of generalized seizure activity of the symmetrical and synchronous spike and wave (SW) variety, and (2) examined changes in the EEG power spectrum prior to SW bursts as prodromal signs which may be used to predict (and ultimately to control) SW bursts. We propose to continue this line of neurobehavioral investigation, using event related potentials of various types as well as other behavioral and physiological tools, to refine further our understanding of the nature of altered consciousness in absence (petit mal) epilepsy. Progress on this part of the protocol has been modest due to a lack of relevant patient subjects, the necessary extensive revision of the hardware (and software) used to record the BAER data, and the very slow development of the necessary software to analyze BAERs during pre-burst periods. These equipment and software needs have now essentially been met and we await appropriate patient subjects.

3. A Taxonomy of Attentional Disorders

The goal of this project is to develop a comprehensive and coherent account of the relation between symptoms of altered or disturbed attention or consciousness as they appear in various clinical entities, the other behavioral and clinical characteristics of the several disorders, and the specific central nervous system damage or disturbance in each disorder. The attentive capacities of the patients will be assessed by a number of measures related to the CPT (continuous performance test) a measure of sustained visual attentive behavior. The ultimate goal will describe the precise attentive deficit (as opposed to cognitive losses) and the nature of the neuropathophysiology associated with each of the following clinical entities: cerebral cortical lesions (frontal, parietal, or temporal lobe); centrencephalic/absence epilepsy; schizophrenia; infantile autism; dementing diseases (Alzheimer's, Korsakoff's, Huntington's); and metabolic diseases (Phenylketonuria, Uremia, Anorexia Nervosa and related illness).

We will attempt, as well, to relate these changes where possible to standardized measures of mnemonic and other cognitive function, and to autonomic indices of attention, arousal, and habituation.

Significance to Biomedical Research and to the Program of the Institute

Since attention disturbance is a characteristic of many significant psycho- and neuropathological disorders, it is essential to have a clear empirical and theoretical account of the role and pathophysiological significance of the symptom. It will aid in understanding the etiology and course of these illnesses and may aid in improving their treatment.

Proposed Course

We have run a small group of schizophrenic, epileptic, and brain-injured patients through our laboratory procedures (i.e., CPT, brain stem auditory evoked potentials, various tests of cognition and memory, autonomic indices of attention, etc.). During the course of the next year, we hope to recruit additional cases from other diagnostic categories into this taxonomic study. With the improved hardware and software now available to us, and with the upcoming visit of Michael Myslobodsky (Visiting Scientist from the University of Tel-Aviv, Israel), we expect to achieve substantial progress during the next year.

We are planning, as well, to prepare for publication two edited books within the coming year; one concerned with petit mal epilepsy and the other with the neuropsychology of attention and attention disorders.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00504-05 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Models in the Monkey of Generalized Seizures of the Absence Type

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Allan F. Mirsky, Ph.D. Chief LPP, NIMH

Others: Eva Bakay Pragay, Ph.D. Research Psychologist LPP, NIMH

Michael Myslobodsky, M.D., Ph.D. Professor, Univ of Tel Aviv Israel

COOPERATING UNITS *(if any)*

University of Tel-Aviv, Israel

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Generalized seizure activity with the electrographic appearance of absence epilepsy (bilaterally symmetrical and synchronous paroxysmal three-per-second spike and wave discharges) can be elicited in the monkey by a variety of methods. These include electrical stimulation of various locations within the brain, injection of convulsant drugs and other substances, and administration of compounds which may alter normal inhibitory mechanisms within the cell. Model seizure states created in these ways are studied in order to test hypotheses about pathophysiological seizure mechanisms, sensory processing and attentional capacities during absence seizures, effects of spike-wave activity on cellular activity, and effects of techniques or maneuvers which may modify or reduce convulsive activity. Most recently this project has involved the following work: we studied the (paradoxical) seizure-inducing effects of a GABA-enhancer and the effects on auditory brain stem evoked potentials of generalized seizures induced by injection of pentylenetetrazol.

Project Description

γ-vinyl GABA and γ-acetylenic GABA are two recently synthesized compounds whose metabolic effects include the blocking of the enzyme action responsible for the metabolism of the inhibitory neuro-transmitter GABA. The accumulation of GABA thus produced should have an anticonvulsant action, and so it does, at moderate doses of these compounds. However, as the dose is increased, there is a paradoxical rebound effect and animals treated with large quantities of either γ-vinyl or γ-acetylenic GABA have shown paroxysmal seizure activity. And of interest to us is the fact that the seizure activity is not the clinically obvious generalized tonic-clonic variety. Instead, although widespread spikes and spike-wave patterns may be seen, there may be few clinical signs. Such an effect is reminiscent of absence seizures (staring spells) in human centrencephalic epilepsy. We are in the process of exploring the utility of these compounds for producing model seizures of the petit mal variety in the monkey.

Significance to Biomedical Research and to the Program of the Institute

This experiment provides information concerning the role of various neurotransmitter substances in consciousness and in generalized seizures and contributes to the current efforts to produce an accurate primate-based model of the pathophysiological processes in absence epilepsy.

Proposed Course

We will be continuing with this experimental program as primate facilities become available to LPP. Together with various intramural and extramural scientists, plans are being developed to publish a book reviewing the recent developments in the biochemistry, electrophysiology and genetics of absence epilepsy. The book will incorporate much of the material germane to this project. Moreover, Dr. Michael Myslobodsky, who will be a Visiting Scientist during the next fiscal year, is contributing to a review article on absence epilepsy being prepared jointly with members of the LPP.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00505-05 LPP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
State Change Effects on Visual Processing and Attention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard K. Nakamura, Ph.D. Senior Staff Fellow LPP/NIMH

COOPERATING UNITS *(if any)*

Laboratory of Neuropsychology, Laboratory of Cerebral Metabolism, and Neuropsychiatry Branch, NIMH

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NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Information Processing in the Monkey Brain: We have been developing a transcortical (surface-to-depth) event-related potential (ERP) method to follow the path of cortical information processing across the monkey brain. The transcortical ERPs will be compared to neural activity in the monkey as well as to ERPs measured from the scalp in both monkeys and humans. Such comparisons will establish the neurophysiological and anatomical basis for the human ERPs that have been shown to be linked to interesting cognitive events.

We map ERP across the brain during information processing of a go/no-go visual discrimination task in the monkey. The ERPs are recorded via transcortical (surface-to-depth) electrodes implanted in sets of up to 37 pairs in each monkey. Space-time images of that activity are then constructed. We have previously reported on the reliability, replicability, sensitivity to task manipulation of the data generated (see 1984 annual report).

Others:

Mortimer Mishkin, M.D.	Chief	LN/NIMH
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William Freed, Ph.D.	Chief, PNS	NPB/NIMH
John Morihisa, M.D.	Chairman, Dept. Psychiatry Veterans Admin. Hospital	Wash., D.C.
Louis Skoloff, M.D.	Chief	LCM/NIMH
Charles Kennedy, M.D.	Guest Researcher	LCM/NIMH
Carolyne Smith, Ph.D.	Chemist	LCM/NIMH
Richard Coppola, D.Sc.	Senior Engineer	LPP/NIMH
Yehuda Salu, Ph.D.	Guest Researcher	LPP/NIMH
Allan F. Mirsky, Ph.D.	Chief	LPP/NIMH

Project Description

1. Relationship of Cortical ERP to Perception

To minimize artifacts related to eye movements or eye position, we have developed a new visual stimulus set that consists of four stimuli of four dots each:

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a. o          b.        o       c. o          d.       o
     o                  o                 o              o
       o              o                 o                o
         o          o                     o            o
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In two of the stimuli (a,b) the four dots form diagonal lines, and in the other two (c,d) the four dots form diamonds with the same dominant angle as the diagonals. The objective for our monkeys is to respond in one way to the diagonals (e.g., go) and another way to the diamonds (e.g., no-go). Brightness, contour, and distance of dots from any fixation point are inherently controlled and no single dot can be used to distinguish between the stimulus categories. The animals must base their responses on the spatial relationship of the dots. In addition, the stimuli are symmetrical so that there is no stimulus-generated inclination to move the eyes from the center of the stimulus presentation screen. With this stimulus set in our task it is possible to evaluate stimulus, stimulus category, motor response, and cortical areas differences in the ERPs.

To date we have found that: (1) ERPs from all electrodes displayed additivity in that the early sensory components generated by the diagonal stimuli (averaged together) were identical to those generated by the diamond stimuli (averaged together) even though the individual stimuli produced highly differentiable results in some cortical areas; (2) all electrodes in modality specific visual areas showed clear stimulus-related differences beginning at about 80 ms in stiate cortex; (3) the differences seen in striate and prestriate cortex could be related to the locations of the dots within the individual stimuli; (4) unlike striate and prestriate placements, two inferior temporal electrodes showed ERP differences between 160 and 200 ms that predicted the animal's motor response though time-locked to the stimulus, and

preceded it by 140-180 ms; (5) contrary to prior reports, none of the visual system ERPs showed differences that were time-locked to the motor response; and (6) electrodes outside of the modality-specific visual areas showed only motor response-related differences, not stimulus-related differences. These differences appeared in prefrontal and parietal areas only after the motor response had begun.

2. Relationship of Cortical ERP to Motor Behavior

We have uncovered two peculiarities concerning the ERP from motor cortex. First, in addition to the motor component itself, there is an early component that reflects the sensory input. This input is time locked to the visual input and occurs earlier than the visual signal seen in visual cortex. Second, we have found that the motor component of the ERP from motor cortex can be disassociated from the motor behavior that we are measuring. The peak of the ERP is only sometimes coincident with the reaction time to movement of the animal. For instance, when we manipulate the ease of the task as in all-go sessions, then the relationship between motor cortex and behavior is uncoupled. This finding conflicts with the generally held views of motor cortex function and raises questions both about these views and about the nature of ERP recording.

3. Relationship of Cortical ERP to Unit and Multiunit Activity

It is important to establish the relationship between the neural activity in the brain and the slow potential activity monitored with ERPs. Although we can justify studying slow potentials merely on the basis of a valid linkage to behavior, an understanding of the relationship to physiology is critical before ERPs can be seriously studied in basic research. We are currently recording both single unit activity and slow potentials from the same recording electrodes. By appropriate filtration, we can compare single unit activity, multiunit activity, and slow potential activity. This appears in a steady record punctuated by behavioral events so we can hope to evaluate overall differences which may be caused by external or internal events.

4. Relationship of Cortical ERP in Monkey to Human Scalp ERP

The discovery that the human scalp ERP registers significant cognitive events has led to an explosion of psychophysiological research. However, the relationship of the various components of the ERP to neural firing or to neural substrates remains poorly understood. Our behavioral task was designed so that it could be performed not only by monkeys but also by humans. This makes it possible to directly compare the results obtained from scalp recordings in humans to those obtained with skull and transcortical electrodes under nearly identical behavioral circumstances in the monkey. In-dwelling bipolar electrodes must provide a more detailed and sensitive picture of activity in the brain than scalp recordings. A promising start here has already been made.

The N100 (N1-P2) wave is a stimulus-triggered, attention-sensitive component of the human scalp ERP that occurs at about 100 ms. Its amplitude

is thought to reflect: (1) when a target can be distinguished from other stimuli on the basis of simple stimulus characteristics, such as location or pitch (audition), and also (2) when the stimulus that has occurred is the target event. It is never seen in an omitted event. There is speculation that this component is the result of a preset filter.

In the monkey, we find a negative-positive component that arises to a visual stimulus in modality-specific visual cortex. The negative peak occurs at about 100 ms and thus corresponds to N100. In striate cortex, its amplitude is only dependent on the physical characteristics of the stimulus. On the other hand, in a later visual processing stage, inferior temporal cortex, the amplitude of this component is dependent both on the physical characteristics of the stimulus and its meaning. Thus, N100 arises from multiple modality specific sources, and its characteristics depend on the summation of these sources.

Another component of the human ERP, the P300 wave, has been shown to be a marker for the conscious processing of behaviorally significant information. It is recorded from scalp leads with the strongest activity appearing over parietal cortex. By manipulating the probability of stimuli in the go/no-go task that our monkeys perform, we have been able to see that a P300-like wave measured from the skull over the parietal lobe gets larger on go trials when those trials become less probable. A careful comparison of the relationship of this P300-like wave to the ERPs from the bipolar cortical electrodes thus far suggests that P300 does not emanate from cortex. This fits with some data from human patients undergoing ERP recording during neurosurgery. In the future, we will obtain ERPs from subcortical placements in monkeys to try to locate the source or sources of the P300 wave.

These results indicate that the spatial resolution of transcortical ERPs in monkeys permit us to subdivide components seen at the scalp in humans as well as provide information on the locus of origin.

Significance to Biomedical Research and the Program of the Institute

We hope to understand the mechanisms underlying information processing, attention, and consciousness in animals and man. This is of fundamental importance in its own right, but will also enable us to develop adequate animal models of human clinical syndromes featuring reduction of attention or consciousness, such as schizophrenia, dementia, and petit mal epilepsy.

Proposed Course

We wish to follow-up on the intriguing results on visual and motor area responses. The suggestions that inferior temporal cortex may be directing the behavior of our monkeys in this task, and that there is an early sensory signal supplied to motor cortex, must be re-examined with appropriate controls.

We intend to modify the task to make it more difficult or more unpredictable to increase the probability that the task will engage cortex more fully and, thus, be more amenable to analysis with cortical electrodes.

We will examine the participation of subcortical structures in the processing of the go/no-go discrimination task. Transcortical ERPs will be compared with those seen from the scalp in humans. This will allow the localization of the sources and may add insight into the mechanisms generating these potentials.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00506-05 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Attention-Related Neurons in the Brain of the Rhesus Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eva Bakay Pragay, Ph.D.	Research Psychologist	LPP/NIMH
Others:	Richard K. Nakamura, Ph.D.	Senior Staff Fellow	LPP/NIMH
	Allan F. Mirsky, Ph.D.	Chief	LPP/NIMH

COOPERATING UNITS *(if any)*

None

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with an analysis of the activity of nerve cells in that system within the primate brain which is necessary and responsible for the process we refer to as attention. Monkeys trained to perform visually-guided go, no-go discrimination tasks are tested whilst extracellular recordings are made from brain regions thought to be part of an attentional system. The most recent study in this series examined structures in the forebrain.

The exploration of the frontal cortex has been completed. Current work investigates attention-related activity in posterior parietal and extrastriate cortices.

Project Description

1. Objective

This project has the following objectives:

a. Extending our research for attention-related units to the posterior parietal and pre-occipital cortex of the monkey brain.

b. Increasing the attention-load of the go/no-go task by introducing variable pre-stimulus waiting periods by shortening the available response time in go trials, by manipulation of reinforcement, and by complete randomization of the go/no-go sequence.

c. Comparing the task-related single unit activity with simultaneously recorded multiple unit activity and slow potentials as well as with separately recorded gross evoked potentials.

d. Examining the relationship between attention (as it may be manipulated by variations of the attention task and reflected by the performmance) and activation (as it may be reflected by the fast EEG components and by heart rate).

2. Methodology

Two rhesus monkeys were trained to perform a go/no-go visual discrimination (attention) task, the modified version of the continuous performance test (CPT versus GAT). One animal was implanted with two chambers over the posterior parietal (PP) and the pre-occipital visual (VC) cortices for single and multiple unit recording and by transcortical macro-electrodes around the periphery of the chambers and some other cortical loci. The other animal is in the final stage of training and will be implanted soon.

ERP Results from Transcortical Electrodes

All ERPs, including those obtained from non-visual areas (motor and prefontal areas) contained an early (less than 100 msec) component and several later components. The early component was similar in go and no-go trials in all areas, and reflected stimulus processing while some later components showed go/no-go differences in some areas and reflected motor processing.

Results from Unit Recording

In 100 penetrations, 80 task-related units were recorded from both chambers and 50 of them analyzed in detail. The task-related units were classified into categories used in previous experiments with the go/no-go task. Units which responded only in go trials were called Type I, while those which responded in both go and no-go trials were designated as Type II. In the present sample, the vast majority in both cylinder areas were Type II (PP=20/27 or 74.1%; VC=21/26 or 80.1%). The Type I units were similar to those seen in the prefrontal cortex: the majority of them were late, that is, responded after the behavioral response. The majority of the Type II units (PP=13/20 or 65%; VC=13/21 or 61.9%) showed

similar ("symmetrical") activity in both go and no-go trials in terms of magnitude, latency, and morphology. On the basis of peak post-stimulus activity alone, even more units could be classified as symmetrical.

Three Type II subclasses could be distinguished: Type II-a units showed post-stimulus depression of activity in both kinds of trials. In Type II-b units, the post-stimulus no-go response was biphasic: the early depression was followed by a post-trial increase. Type II-b units were asymmetrical by definition, while the other subtypes could have a symmetrical and an asymmetrical variant. Type II-c units showed post-stimulus increase in both go and no-go trials.

The go/no-go asymmetry may be due, in part, to the asymmetrical reinforcement used here in the basic version of the task. This relationship was demonstrated in some units which became more similar in their go and no-go responses if a symmetrical reinforcement was introduced.

A substantial number of Type II units (PP=9/20 or 45%; VC=8/21 or 38.1%) showed anticipatory activity in the pre-stimulus waiting period. These units responded to the variation of the pre-stimulus period the same way as brainstem RF and anterior frontal units, in that a prolonged waiting period increased anticipation while a shortened waiting period showed that fixed waiting periods decreased or abolished it. Comparison of varied versus fixed waiting periods are not indispensable for developing anticipation (at least within the 1s-2-3s range).

In general, the most conspicuous property of both PP and VC populations was the predominance of post-stimulus inhibition. In the majority of cases, the unit was most active in the intertrial interval and least active following the stimulus onset. This was more true for the PP than the VC populations.

Reaction time and heart rate were useful and meaningful indices of attention-related central activation. Reaction time was shorter following the prolonged pre-stimulus waiting period and it was shorter in asymmetrical reinforcing conditions. The attentional character of the underlying increased activation was manifest in decreasing heart rate under these conditions. On the other hand, the decreased reaction time due to elimination of choice (all go series) was not accompanied by deceleration of heart rate.

Area Characteristics and Differences

Considering the relatively small sample studied, and considering the lack of histological verification, it would be premature to state area differences. However, some general observations may be made. The two areas (PP and VC) showed more similarities than differences, which indicates a considerable territorial overlap between them. Both populations contain a considerable number of units which are similar to those seen in other non-specific areas. Type II units are responsive to manipulation of attention in terms of focusing in time (preparation-anticipation of behaviorally significant stimuli). They also respond to the manipulation of reinforcement contingencies. In terms of within-chamber differences, the most anterior part of the PP chamber has a population which seems to be related to the movement of the contralateral hand and arm. The response of these units is particularly late; the activity begins long after the behavioral

response. It is likely that this part of the chamber is reaching into the somatosensory cortex. This assumption is supported by the latency and configuration of the gross evoked potentials obtained near to that area (see above).

This is the only correlation between single unit and gross ERP profiles. In other respects, the discrepancies are more conspicious. This is especially true for the presence of early components in all ERP placements and the rarity of early "on" responses in the single unit population. The differences may be explained or attenuated by the analysis of the multi-unit records obtained through the microelectrode. This analysis is awaiting the appropriate computer program.

Significance to Biomedical Research and to the Program of the Institute

Our study is relevant to three major issues: (1) the localization of attention; (2) the functional organization of the posterior association cortex; and (3) the comparison of single unit activity with multi-unit activity and with gross evoked potentials. The study of attention is important because attention deficit is a characteristic symptom in many psychopathological and neurotic conditions. Moreover, comparison of various kinds of evoked responses may lead to a better understanding of the neurophysiological sources of gross evoked potentials which are important tools in understanding attention deficit in humans.

Proposed Course

This project will terminate with this report.

Publications

Mirsky, A.F. and Bakay Pragay, E. Brain mechanisms in the processing of sensory information: Clinical symptoms, animal models, and unit analysis. In D.E. Sheer (Ed.), Attention: Theory, brain functions, and clinical application. Hillsdale, N.J., Erlbaum, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00508-03 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropsychological Evaluation of Psychiatric and Neurological Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Connie C. Duncan, Ph.D. Chief, Unit on Psychophysiology LPP, NIMH

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, Laboratory of Clinical Science, NIMH; Medical Neurology Branch, Developmental and Metabolic Neurology Branch, NINCDS; Albert Einstein College of Medicine; and Chestnut Lodge Hospital

LAB/BRANCH

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A comprehensive neuropsychological test battery has been devised to provide a complete assessment of various cognitive and sensory functions that can be related to damage or dysfunction in different regions of the brain. The battery comprises tests designed to tap the following aspects of behavior: executive functions, language, attention, visual-spatial capacity, memory, and motor behavior. In addition, measures of psychometric intelligence, personality, visual acuity, color vision, and hand and eye dominance are included. The battery provides a thorough assessment of the neurobehavioral capacities of the various categories of patients who are studied by investigators in the LPP. The data thus provide a complete behavioral profile against which to relate the neurophysiological, neuroradiological, and biochemical information that is gathered concurrently on these patients. The data can also provide neurobehaviorally-defined subgroups aimed at reducing variability in psychiatric diagnosis, treatment, and outcome.

Other Professional Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Emile Brouwers, Ph.D.	Visiting Associate	LPP, NIMH
Robert Post, M.D.	Chief	BPB, NIMH
David Jimerson, M.D.	Chief	SBP, LCS, NIMH
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Roger Porter, M.D.	Chief	MNB, NINCDS
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John Fink, M.D.	Clinical Associate	DMNB, NINCDS
Elkhonon Goldberg, Ph.D.	Associate Professor of Psychiatry	Albert Einstein College of Medicine
C. Wesley Dingman, M.D.	Assistant Clinical Director	Chestnut Lodge Hospital

Objectives

This project has as its goal the investigation of neurobehavioral functioning in neuropsychiatric patients, such that: (1) Neuropsychological profiles of diagnostically distinct groups can be obtained, and differences in the profiles between groups can be used as an indication of specific organic influences in the psychopathology of these patient groups; (2) Neuropsychological profiles of patients within a heterogeneous diagnostic classification can be obtained. Differences between patients can provide neurobehaviorally-defined subgroups that might reduce variability in diagnosis and treatment, as well as improve outcome; and (3) The obtained comprehensive neurobehavioral data can be correlated with neurophysiological, biochemical, and neuroradiological data that are being collected concurrently on these patients to provide a link between pathophysiology and behavior.

Methods Employed

The neuropsychological battery includes the tests listed below. Cognitive and sensory functions are presented in tabular form along with the test(s) used to assess them. Administration of the battery takes 8-12 hours.

FUNCTION MEASURED	TEST
Executive	
Sequencing, Attention	Trail Making Test
Attention	Stroop Color-Word Test
	Talland Letter Cancellation Tests
Perception and Reasoning	Raven's Progressive Matrices
Concept Formation and Abstraction	Wisconsin Card Sorting Task
	Halstead Categories Test
Language	
Initiation	Verbal Fluency Test
Lexical	Boston Aphasia Test--Word Discrimination and Visual Confrontation Naming

Written	Boston Aphasia Test--Word Discrimination
Comprehension	Token Test Goldberg's Semantic Test
Auditory Discrimination	Wepman Auditory Discrimination Test
Oral Apraxia	Boston Aphasia Test--Oral Agility
Vigilance	Continuous Performance Test
Visual-Spatial	Hooper Visual Organization Test Witkin's Embedded Figures Test Butter's Embedded Figures Test
Memory	
Global	Wechsler Memory Scale
Recent Verbal Memory	Buschke Selective Reminding Test Rey Auditory Verbal Learning Test Babcock Story Recall Test
Remote Verbal Memory	Boston Remote Memory Test
Recent Visual-Spatial Memory	Kimura's Recurrent Figures Test Rey-Osterreith Complex Figure Test
Motor Functions	Purdue Pegboard Boston Apraxia Test
General Intelligence	Wechsler Adult Intelligence Scale-Revised or Wechsler Intelligence Scale for Children-Revised
Personality	Minnesota Multiphasic Personality Inventory
Sensory and Perceptual	Visual Acuity Color Vision Stereopsis Test Hand Dominance Eye Donimance

Major Findings

The test battery or parts thereof has been administered to a total of 145 subjects, of which 42 were control subjects (16 males; 26 females).

1. Eating Disorder Patients

Testing has continued on patients with eating disorders and matched, normal controls. Preliminary comparisons of 13 underweight anorexic and 16

normal-weight bulimic patients, as well as 9 long-term weight-restored patients with anorexia nervosa revealed that on a test of general intelligence (WAIS-R), the three groups did not differ in Full Scale IQ, Performance IQ, or Verbal IQ. All obtained mean IQ scores were in the average range and did not differ significantly from 100. However, the underweight anorexics performed significantly worse on the Comprehension Subtest than either the bulimics or the long-term weight-restored anorexics ($p < .001$ and $p < .05$, respectively); whereas the difference between the latter two groups did not reach statistical significance ($p > .20$). Other subtests failed to reveal significant differences. In addition, underweight anorexic patients had significantly lower scores on tests of perceptual matching and concept formation using novel, nonverbal stimuli. Their performance on these tests as well as on some memory tests, however, improved significantly following weight restoration. The personality profile (as assessed by the Minnesota Multiphasic Personality Inventory) of patient groups with anorexia appeared abnormal. The profile of long-term weight-restored anorexics did not differ from matched, normal controls; however, both the underweight and the short-term weight-restored anorexics showed significant differences from the other two groups but did not differ from one another. Specifically, they showed elevated scores on the Depression, Schizophrenia, Psychasthenia, and Psychopathic Deviate scales. Patients with bulimia showed similar elevated scores on these scales but, in addition, showed an elevated score on the Paranoia scale.

2. Affective Disorder Patients

A project is underway on patients with affective illness; the aim is to assess neuropsychological performance as a function of drug treatment (placebo, lithium, and carbamazepine). Specifically, the different mechanisms of action of these two drugs (e.g., on vasopressin) may enhance or impair various aspects of neuropsychological functioning. Thus, it could provide further data on the effects of this neuropeptide on cognitive and mnemonic functioning as well as other aspects of information processing. In addition, since vasopressin abnormalities have been reported in patients with eating disorders, comparisons will be made between those cases and patients with affective disorders. A subset of tests from the battery that tap various aspects of attention, verbal and visual-spatial memory, problem solving ability, motor skills, personality, and the ability to estimate time were selected. These tests were chosen specifically because they are repeatable or have parallel forms. This "mini-battery" has been administered to 22 patients with affective illness; in nine cases, one retest under a different drug condition is available. In four cases, a second retest is available. In addition, an attempt is made to counterbalance the mood state of the patient at the time of testing. However, since the number of data points in some cells of the design is extremely small, no data analyses have been performed to date.

3. Acquired Immune Deficiency Syndrome Patients

A 4 to 5-hour battery was selected to test a wide range of cognitive functions, including memory, concentration, and executive functions as well as mood in patients with Acquired Immune Deficiency Syndrome (AIDS). Thirteen

patients and 10 matched homosexual control subjects have been tested. Psychological testing using the same battery on a group of homosexual patients with chronic, active hepatitis is currently underway. This is being conducted by the staff of the Biological Psychiatry Branch, NIMH. Preliminary data analysis contrasting the AIDS patients with the non-medical control subjects revealed a significant deficit in concentration and execution speed for the AIDS patients. This could be related to the scores on the Depression scale of the MMPI, which were also elevated. In addition, the AIDS patients showed an unexpected relative deficit on tests measuring old learning and semantic verbal knowledge. The AIDS patients and the controls are to be retested shortly, using a subset of the initial battery, complemented with psychological tests indicated by the results of the preliminary analyses.

4. Gaucher's Disease Patients

We have initiated a project to study the neuropsychology of Gaucher's disease, an inherited metabolic disorder of lipid metabolism. Three clinical categories of this disease can be distinguished: Type 1, the adult, chronic, non-neuropathic form, which can be manifested any time from birth to old age; Type 2, the infantile form, which has a fast deteriorative course, with death usually occurring before two years of age; and Type 3, the juvenile or subacute neuropathic form, which usually occurs in childhood and has a slow deteriorative course. A major difference between Types 1 and 3 of Gaucher's disease is the presence of central nervous system (CNS) pathology and associated clinical signs. While CNS involvement in Type 3 is well-established, there are some reports of CNS-signs (dementia) in Type 1, but these findings have been observed exclusively in non-Jewish patients. We are investigating the intellectual and cognitive performance of Type 1 non-Jewish, Type 1 Jewish, and Type 3 Gaucher's patients between the ages of one and 30 years to study the occurrence of intellectual deterioration, and, if present, the rate of deterioration. Also studied are differences in the neuropsychological profile among groups as well as the presence of focal signs. A subset of the battery has been selected and will be administered on an annual basis to monitor the performance of these patients. Five patients have been evaluated to date.

Significance to Biomedical Research and the Program of the Institute

These investigations contribute to the basic understanding of the development and organization of structure-function relations in the brain. They represent an empirical approach to identifying neuropsychological deficits that may be shared by, or differentiate among, various psychopathological and neurological disease processes. These observations will be related to psychophysiological, neuroradiological, and biochemical data in order to develop a better understanding of the factors underlying psycho- and neuropathology.

Proposed Course

We plan to continue testing patients studied by investigators in the LPP as well as to increase our sample of normal controls, to the extent that

resources and time permit. As the sample size increases, we will be able to contrast the neuropsychological test profiles of the different clinical groups under study, using a broad representative sample of normal controls. Furthermore, we plan to begin to interrelate this behavioral information with neurophysiological, biochemical, and neuroradiological information obtained on the same samples. We also plan to evaluate schizophrenic patients, when they are actively symptomatic and when they are in remission, and to study the relatives of schizophrenic patients, as these subjects become available.

Publications

Brouwers, P., Riccardi, R., Fedio, P., and Poplack, D. Long-term neuropsychological sequelae of childhood leukemia: Correlation with CT brain scan abnormalities. Journal of Pediatrics, 1985.

Brouwers, P. Neuropsychological abilities of long-term survivors of childhood leukemia. In J. Beckmann (Ed.), The quality of life of cancer patients. Raven Press: New York (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00509-03 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Attention Disorders As Assessed by Event-Related Brain Potentials

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Connie C. Duncan, Ph.D., Chief, Unit on Psychophysiology, LPP, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Clinical Science, Neuropsychiatry Branch, Child Psychiatry Branch, NIMH; Medical Neurology Branch, Developmental Neurology Branch, NINCDS; Laboratory of Neuroscience, NIA; and Chestnut Lodge Hospital

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	1.9	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to investigate the roles of event-related brain potentials, attention, and information processing and their interrelationships in the etiology, pathology, and prognosis of psychiatric and neurological disorders. Major emphasis is on the diagnostic specificity of disorders of attention and cognition and identification of the specific aspects or stages of information processing underlying observed decrements in performance. Concurrently recorded event-related brain potentials and performance on cognitive tasks are used to define mechanisms of cognitive failure in subjects with diagnoses of schizophrenia, seizures, attention deficit disorder, learning disorders, eating disorders, and dementing diseases. Biological processes influencing event-related brain potential activity are investigated by testing the effects of drugs and by correlating these variables with biochemical measurements. Psychological correlates are investigated by relating the data to extensive neuropsychological, psychiatric, and personality measures as well as performance on behavioral tasks.

Other Professional Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Walter H. Kaye, M.D.	Staff Psychiatrist	LPP, NIMH
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John M. Morihisa, M.D.	Chairman, Dept. of Psychology, Veterans Administration Hospital	Washington, D.C.
William H. Theodore, M.D.	Staff Neurologist	CES, MNB, NINCDS
Judith M. Rumsey, Ph.D.	Senior Staff Fellow	CPB, NIMH
Martha B. Denckla, M.D.	Chief	ABD, DNB, NINCDS
Stanley I. Rapoport, M.D.	Chief	LN, NIA
James V. Haxby, Ph.D.	Senior Staff Fellow	LN, NIA
C. Wesley Dingman, M.D.	Assistant Clinical Director	Chestnut Lodge Hospital

Project Description

A. Objectives

The major objective of this project is to yield data that will illuminate the neuropsychological bases of the cognitive and attentional deficits in the clinical disorders of schizophrenia, eating disorders, epilepsy, dyslexia, dementia, and other forms of brain pathology. Defining the specific ways in which information processing can fail may provide new diagnostic strategies for more effective evaluation and treatment of patients with attentional and cognitive impairments. A related objective of this project is to differentiate state versus trait attributes of these disorders to increase understanding of their etiologies. Concurrently obtained event-related brain potentials (ERPs) and measures of performance during active cognitive processing are used to define the mechanisms of attention failure in these syndromes. Defining and understanding the different determinants and forms of attentional and cognitive failure is diagnostically important as well as useful in characterizing the nature of the psychobiology of attention disorders. Finding differences in overall response levels between normal subjects and patients is a necessary first step; however, the goal is to use the knowledge to lead to new approaches to classification, treatment, and etiology.

B. Methods Employed

1. Electrophysiological Assessment

The general methods of these studies include recording the EEG, utilizing the International 10-20 system of electrode placement, while trains of stimuli are presented to the subject. Stimulus presentation and data collection are controlled by a PDP-11/34 or PDP-11/73 computer. The EEG is averaged to yield ERPs. Since these electrical waves are associated in time with either an event in the environment, such as the presentation of stimulus, or with an internal cognitive event, they are called event-related brain potentials.

Brainstem auditory evoked responses (BAERs) are used to measure, directly and noninvasively, the progress of a sensory signal through brainstem to the cortex, and thereby obtain a measure of the integrity of brainstem functioning. BAERs are obtained by presenting click stimuli to the ears.

Somatosensory evoked potentials (SEPs) provide data on conduction time as well as the magnitude of response in peripheral nerves, the spinal cord, brainstem, and cerebral cortex. They also serve as a reliable index of proprioceptive function and the functional integrity of the somatosensory pathways. SEPs are generated by separately stimulating the fingers, the median, ulnar, or radial nerve at the wrist, the tibial nerve at the ankle, or the peroneal nerve at the knee.

Evaluation of endogenous components of the ERP, associated with higher-level processes such as selective attention, learning, memory, and decision-making, yield information on the attentional and cognitive functioning of the subject. The ERPs are elicited by trains of auditory, visual, or somatosensory stimuli presented in the context of an attentional or cognitive task. The selection of tasks, which use reaction time techniques as well as recall and recognition of stimulus material, allows for the measurement of a pattern of cognitive behaviors and associated ERPs, where different components define different aspects of information processing. Using ERPs, it is possible to get an indication of the subject's processing of all environmental stimuli, both relevant and irrelevant, and thus to assess, for example, the differential processing that is the hallmark of selective attention.

A major focus of our investigations is the "P300" component of the ERP. This scalp-derived electrical potential appears 300-900 milliseconds after an event that engages the interest or attention of a subject and is a positive voltage as recorded on the scalp; hence the name P300. The amplitude of the P300 component depends on the amount of processing capacity invoked by a stimulus and reflects stimulus evaluation and decision-making activity. It is also a sensitive indicator of orienting reactions to novel, surprising, or incongruous stimuli and a predictor of the memorability of events. Moreover, P300 allows a direct evaluation of the subjective probabilities that a subject assigns to event outcomes and may reflect the extent to which a stimulus is encoded. The latency of P300 indexes the time required to classify and evaluate a stimulus independent of response-production factors. ERPs can thus help to clarify the timing and order of neural events in information processing activities and to identify the aspects or stages of information processing responsible for observed decrements on cognitive tasks in a variety of clinical populations.

2. Neuropsychological Assessment

Normal volunteers are screened by a psychologist who uses the lifetime version of the Schedule for Affective Disorders and Schizophrenia to exclude those with a past or current history of psychopathology. Most patients and normal volunteers are evaluated on an extensive neuropsychological battery of cognitive and sensory functioning. When appropriate, tests of formal thought

disorder are administered. We plan to correlate the neuropsychological and electrophysiological data to aid in the classification of disorders characterized by attentional deficit and cognitive failure.

3. Biological Assessment

In collaboration with other laboratories, subjects are assessed for drug treatment responsiveness. Blood, urine, and cerebrospinal fluid measurements reflecting neurochemical activity are correlated with electrophysiological and neuropsychological data. We also plan to use X-ray transmission tomography (CT scan) to measure ventricular size. These data will be correlated with electrophysiological data to yield information on the relation betweeen ERPs and cerebral structures.

Major Findings

We are using a battery of attentional paradigms, which tap visual and auditory information processing systems, to investigate patients with schizophrenia, absence epilepsy, eating disorders (anorexia and bulimia), adult dyslexia, and progressive idiopathic dementia. The battery provides a differential assessment of specific types of attention, including the ability to initiate, select, inhibit, shift, and sustain attention. The protocol also includes evaluation of automatic and effortful cognitive processes. The rationale for the approach of using a battery of tasks that tap specific cognitive processes is to allow inferences about which processes are uniquely impaired in one group in comparison to other groups. To determine whether ERPs can serve as sensitive yet specific markers of disorder, patients with diverse symptomatologies and diagnoses are compared. The ERP measures are correlated with concurrently recorded behavioral responses, including reaction time, as well as performance on tests from the LPP Neuropsychological Battery.

Preliminary analyses indicate that the P300 is reduced in amplitude in schizophrenic and dyslexic patients as compared with normal subjects, an effect that increases with increasing task demands. Because of the small number of subjects tested, the reliability of this result is uncertain at present. Moreover, additional normal control subjects are needed. The data do, however, suggest altered cerebral mechanisms underlying attentive behavior in schizophrenia and adult dyslexia.

Persons with eating disorders appear to be characterized by altered cognitive processing. In particular, there have been reports that patients with anorexia nervosa may be impaired in automatic but not in effortful processing. We are using ERPs to assess these aspects of information processing. The results to date indicate separation on the ERP measures between anorexic and control and between anorexic and bulimic subjects. Specifically, anorexic patients appear to show disturbances in automatic processing, as indexed by a component reflecting an automatic cerebral mismatch process. Altered effortful processing, as measured by P300 amplitude, is also seen in the anorexics; the difference increases with increasing task demands. Bulimics resemble controls on most measures. Preliminary findings after weight restoration in the anorexic patients indicate reversal in some, but not all, of the ERP abnormalities.

We are currently conducting a dose-response study of the alpha-2 adrenergic agonist clonidine in normal volunteers. Electrophysiological, neuroendocrine, and behavioral function are assessed following intravenous administration of clonidine and placebo. Preliminary results indicate that the ERP indexes dose level in a more or less linear fashion. Such a pattern of results suggests that the ERP could potentially serve as a neurophysiological assay of activity in the locus coeruleus nucleus.

Significance to Biomedical Research and the Program of the Institute

Since attentional deficit and cognitive dysfunction are characteristic of many psychopathological and neuropathological disorders, it is important to develop a precise empirical and theoretical account of these symptoms. The scalp-recorded ERP is the only noninvasive technique available for studying rapidly changing neural activity associated with cognitive processing in human subjects. The ERP provides information on mental events involved in selective attention, stimulus evaluation and decision-making, memory, learning, and response preparation. The temporal resolution of ERPs can support inferences about brain activity on time scales not possible in studies using tissue assays or radioactivity. Because of the noninvasive character of P300, patient state can be monitored often enough to assess the effects of specific clinical or experimental variables. The appropriateness of evaluating ERPs in studies of attention is apparent, as they may provide a dissection of the various components involved and thereby permit more precise identification of the types of information processing deficits responsible for poor performance on attention tasks in a variety of patient groups. It is hoped that the developing battery of ERP and neuropsychological tests applied to patients characterized by attentional deficit and cognitive dysfunction will ultimately provide a neurobiological profile of each disorder and lead to more refined subcategorizations, as well as to more efficacious treatments.

Proposed Course

We plan to continue collecting data on patients with schizophrenia, absence epilepsy, eating disorders, adult dyslexia, and presumed Alzheimer's disease. In addition, we are planning to implement studies of children with attention deficit and learning disorders and patients with severe anxiety disorders. Our work is aimed at illuminating the neuropsychological bases of the cognitive and attentional deficits in these patient groups. As our sample sizes increase, we plan to evaluate our ERP findings in relation to neuropsychological, behavioral, and biochemical measurements. Of interest are the relation of ERP variables to diagnosis, diagnostic symptomatology, severity of disorder, degree of formal thought disorder, performance on tests of attention, memory, and intellectual functioning, degree of improvement during treatment, and improvement on specific treatments. We plan to expand our investigation of the interrelation among ERP components and neurochemical variables. To increase our understanding of the etiology of schizophrenia, we are planning studies to differentiate state versus trait attributes of the disorder. The strategy we intend to use is to compare normal controls with schizophrenic patients when they are actively symptomatic and when they are in remission. Selected studies with brain-injured cases are planned to test

hypotheses derived from various clinical groups concerning the involvement of brain structures in the pathophysiology of psychiatric disorders. Electrophysiological predictors of clinical response to neuroleptic medications will be sought, as patient availability allows.

Publications

Duncan-Johnson, C. C., Roth, W. T., and Kopell, B. S. Effects of stimulus sequence on P300 and reaction time in schizophrenics: A preliminary report. In R. Karrer, J. Cohen, and P. Tueting, (Eds.), Brain and Information: Event Related Potentials, (pp. 570-577). New York: The New York Academy of Sciences, 1984.

Duncan-Johnson, C. C. P300 applications to research on schizophrenia. Electroencephalography and Clinical Neurophysiology, in press.

Roth, W. T., Duncan-Johnson, C. C., Pfefferbaum, A., and Timsit-Berthier, M. Applications of cognitive ERPs in psychiatric patients. Electroencephalography and Clinical Neurophysiology, in press.

Mirsky, A. F., Duncan, C. C., and Myslobodsky, M. Petit mal epilepsy: A review and integration of recent information. Journal of Clinical Neurophysiology, in press.

Mirsky, A. F. and Duncan, C. C. An introduction to modern techniques of clinical neuropsychology. In T. N. Wise (Ed.), Advances in Psychosomatic Medicine, in press.

Mirsky, A. F. and Duncan, C. C. Etiology and expression of schizophrenia: Neurobiological and psychosocial factors. Annual Review of Psychology, 37, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02235-01 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Topographic Analysis of Brain Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard Coppola, D.Sc. Senior Engineer LPP/NIMH

COOPERATING UNITS *(if any)* Laboratory of Cerebral Metabolism, Neuroscience Branch, Biological Psychiatry Branch, Neuropsychiatry Branch, Laboratory of Clinical Sciences, and Child Psychiatry Branch, NIMH; Epilepsy Branch, NINCDS; Laboratory of Clinical Studies, NIAAA; Laboratory of Neuroscience, NIA

LAB/BRANCH

Laboratory of Psychology and Psychopathology

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NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Electrical brain activity, as an index of central nervous system function, is studied across a range of patient groups with neurological and psychiatric disorders as well as normal volunteers. Using electrophysiological data quantified from event-related potentials and spectrum analysis of EEG recordings, computer-derived brain images are able to provide information about neurophysiological function relating to both cognition and clinical state. Topographic maps efficiently characterize spatial and temporal patterns of brain activity allowing the ability to study the dynamic interaction among brain regions and their relation to function.

The project has two main purposes. The first is to refine the topographic and quantitative analysis methods and establish normative data for various conditions and activation procedures. For example, normal subjects differ with respect to their major focus of resting EEG alpha rhythm; one group shows a dominant parietal locus and one an occipital locus, depending on the alpha frequency.

The second purpose is to apply these methods to the characterization of clinical groups and pharmacological response. Work in progress includes characterization of subgroups of Alzheimer's patients, localization of abnormality in epilepsy patients, localization of drug activation and study of psychiatric patients on various neuroleptic drugs.

OTHER PROFESSIONAL PERSONNEL

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Herbert Weingartner, Ph.D.	Research Psychologist	LPP, NIMH
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Stanley I. Rapoport, M.D.	Chief	LNS, NIA
Susan R. Rose, M.D.	Medical Officer	DEB, NICHD
Harold Sachheim, Ph.D.	Columbia University	New York
Richard D. Weiner, M.D., Ph.D.	VA, Duke University	N. Car.
Victor Milstein, Ph.D.	Carter Memorial, Indianapolis, Indiana	
Werner Herrman, M.D.	AFB, Berlin	W. Germany

Objectives

The overall goal of this project is to develop and apply methods for utilizing the electrical activity of the brain as a measure of central nervous system function with the expressed purpose to study human information processing, including attention, sensory processing, and cognition and to study functional states as seen during chronic or transient conditions. Methods have been developed to display topographic maps that efficiently characterize brain activity in terms of both spatial and temporal patterns. The ability to study these patterns in a dynamic fashion will yield a better understanding of the interaction among brain areas and their relation to function.

The project has two main thrusts. The first is to refine the topographic and quantitative analysis methods and establish normative data for the patterns of brain activity seen in a variety of conditions and under various activation procedures. Differentiation of EEG patterns associated with cognitive and attention-related parameters are of importance to understand the underlying neurophysiological basis of both normal and abnormal brain function.

The second thrust is to utilize these methods and normative base to discover and describe characteristic brain activity patterns in a variety of patient groups. The main hypothesis is that certain patient groups will exhibit regional localization of EEG abnormalities. It is expected that quantitative topographic analysis will provide better sensitivity for this localization than the usual clinical EEG recordings. An additional hypothesis

is the expectation of changes in quantitative EEG parameters with treatment in patient groups.

Methodology

Multilead scalp recordings are made during baseline resting conditions and during various activation procedures. Quantitative reduction of this data is performed by computer spectrum analysis to provide a profile of the energy in the different frequency bands of the EEG. Combining this data with an equal area projection of the scalp surface gives a computer-generated display of a map of brain activity. The map is used to define a baseline condition and changes in the map are used to assess regional patterns during activation procedures. Maps of the raw EEG itself are used to follow the temporal and spatial development of specific EEG events such as a spike and wave complex. Event-related potentials (ERPs) are collected to visual pattern stimulation. Maps are made in a similar fashion for this data and used to determine the intactness of sensory pathways.

Methods employed in specific clinical studies fall into two categories. The first type is where a patient is seen only once. In this case, comparison with other clinical groups or normative data is used to derive characteristic topographic profiles. Correlation or subtyping, using neuropsychological assessment from other studies, may also be carried out. In the second case, patients are seen more than once and assessment is made in regard to change in clinical state, medication, or other treatment.

Collaborative Centers

Because of considerable interest in the research community and as a means to refine these methods and expand the available data base, several collaborating laboratories are now using the system we have developed. This includes laboratories in NINCDS and NIAAA, as well as several outside the NIH. A complete laboratory at St. Elizabeth's Hospital has been set up in the Clinical Neuropsychiatry Section. This collaboration will allow the collection of combined EEG and regional cerebral blood flow data.

An electrophysiology capability, duplicating the one in LPP, has been set up in the Clinical Brain Imaging Section, LCM, NIMH, for combined EEG-imaging and PET studies.

Major Findings

1. Methodology

A major cause for concern in topographic mapping has been the choice of reference electrode. We have shown that forehead, linked ears, common average, and source derivation all yield different maps. We have developed re-referencing methods and the use of a Laplacian transform to produce a reference-free, source-density map that might overcome these problems. Overall, a continuous EEG mapping system has been refined to produce reliable images of brain activity.

A set of activation procedures for simple vigilance and a verbal and spatial memory task have been developed. Data collection is under way to determine if differential hemisphere loading is verified from these last tasks.

2. Normative Studies

Normal volunteers may be classified into two subgroups based on the spatial pattern of their resting alpha rhythm. Those subjects with peak alpha frequency below 10.2 Hz have a parietal pattern and those above 10.2 Hz an occipital pattern. This does not appear to be just a simple spatial difference due to frequency, because the map for the band 10.5 to 12.5 Hz for subjects with peak below 10.2 Hz is also parietal. This suggests two different generators in the normal population. It is unclear, as yet, as to whether or not these differences reflect salient neurophysiological or neuropsychological characteristics of the subjects.

3. Pharmacological Characteristics

An area of activation of beta EEG activity in temporal regions has been demonstrated during IV procaine infusion. A beta activation measure was found to correlate significantly with a behavioral measure of dysphoria.

A pilot study of a double blind crossover of placebo, amitriptyline, chlorepromazine, and diazepam has been completed. There appear to be specific regional effects in addition to the usual spectrum differences due to these drugs.

4. Epilepsy

Recordings from more than 40 seizure patients have been completed. Some characteristic patterns have emerged from the variety of disorders represented. The dynamic pattern of the spike and wave complex was seen to be almost identical in the four petit mal patients available. In all cases, the spike has a mid-line frontal maximum that does not shift position during the time course of the spike itself.

A complex partial case was noted where the combination of mapping and quantitative analysis clearly revealed a chronic abnormality that could be recorded even in the absence of any interictal spiking. The next step will be to follow this abnormality with changes in medication.

Significance to Biomedical Research and to the Program of the Institute

Electrical activity recorded at the scalp is currently the only non-invasive technique available as a window on the physiological functioning of the human brain. While EEG is a very indirect measure of neural activity, its advantage is the ability to reflect changes on a millisecond-by-millisecond basis. This allows EEG to be related to behavior in an ongoing manner. In contrast, PET images have higher resolution and more directly measure neural activity but reflect the summation of activity over a period of time. Localization of function requires not only resolution but the

correlation of activity with behavior. EEG imaging gives complementary data to the other modalities of cerebral metabolism (PET) and blood flow (RCBF).

Proposed Course

1. Methodology

Refinement of the topographic and quantitative methods will continue, especially with regard to the assessment of localization techniques and comparability of data among laboratories. It is also important to develop statistical methods that are appropriate for image data. This is necessary both for the characterization of patterns of activity as well as for between-group comparisons.

Because the human studies are confined to scalp data, primate studies will be useful to verify the ability to localize activity using this technique.

2. Clinical

In Alzheimer's patients, we already know that EEG patterns deteriorate with disease; however, it will be useful to determine if there are patterns of deterioration that vary with subtypes of cognitive impairment or with degree of impairment.

In epilepsy studies, we will be investigating changes with medication as well as pre- and post-surgery evaluations. We also wish to determine whether the seemingly normal EEG from spike-free episodes actually has a pattern variation from control data and/or from the opposite hemisphere for lateralized foci.

We will continue to expand the number of subjects in the variety of clinical studies so that statistical analyses can verify regional differences. Studies under way include: schizophrenic patients on and off a variety of neuroleptic drugs, learning disabled individuals, Parkinson's patients, and moderately impaired Alzheimer's patients.

Publications

Coppola, R. EEG Imaging of brain activity: Methods and potentials. In A. Duerinckx, M. H. Loew, and J. Prewitt (Eds.), Proceedings International Symposium on Medical Images and Icons. IEEE Press, 1984, pp. 222-223.

Coppola, R. Issues in topographic analysis of EEG activity. In F. Duffy (Ed.). Progress in Topographic Mapping of Neurophysiological Data. Butterworths, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02288-01 LPP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Etiological Factors in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	Paul Wender, M.D.	Prof Psychiatry, Univ. of Utah
	Kenneth Kendler, M.D.	Assoc Prof Psychiatry, Med. College of Virginia
	Bjorn Jacobsen, M.D.	Assoc Prof Psychiatry, Univ. of Copenhagen
	Fini Schulsinger, M.D.	Prof. Psychiatry, Univ of Copenhagen
	Dennis Kinney, Ph.D.	Asst Prof Psychiatry, Harvard University
	Loring Ingraham, Ph.D.	Staff Fellow, LPP, NIMH

COOPERATING UNITS *(if any)*

Psychological Institute, Copenhagen, Denmark; Medico Social Research Board, Dublin, Ireland; McLean Hospital, Belmont, Mass.; University of Utah; Medical College of Virginia; Harvard University

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of the occurrence of mental illness in families have been useful in identifying familial forms of the illnesses and in the development of hypotheses regarding the form and strength of genetic and environmental factors in etiology. Where these major variables are separated by the process of adoption, specific etiologic hypotheses can be tested separately and in combination. A total national sample of 14,500 adult adoptees in Denmark, including 76 who have developed schizophrenia, provide the basis of one phase of this research; the other phase is represented by schizophrenic patients and their families residing in Roscommon County, Ireland, where the prevalence of schizophrenia appears to be three times higher than its prevalence in England and other Western countries.

Project Description

Studies of the occurrence of mental illness in families have been useful in identifying familial forms of the illnesses and in the development of hypotheses regarding the form and strength of genetic and environmental factors in etiology. Where these major variables are separated by the process of adoption, specific etiologic hypotheses can be tested separately and in combination. A total national sample of 14,500 adult adoptees in Denmark, including 76 who have developed schizophrenia, provide the basis of one phase of this research; the other phase is represented by schizophrenic patients and their families residing in Roscommon County, Ireland, where the prevalence of schizophrenia appears to be three times higher than its prevalence in England and other Western countries.

The objective of the Danish adoption study is to extend the survey, initially confined to the city and county of Copenhagen, to all of Denmark, evaluating the strength of genetic and family-related environmental influences, and to define more explicitly the traits which comprise the syndrome of latent schizophrenia. The aim of the study in Roscommon County is to determine the proportion of familial versus sporadic forms of schizophrenia, comparing the proportions in Ireland and Denmark, to indicate whether the marked increase in prevalence of schizophrenia in Ireland is attributable to family-related influences or environmental influences unassociated with the family.

1. The Danish Adoption Study

Comprehensive interviews with the biological and adoptive relations of 34 schizophrenic adoptees and 34 non-schizophrenic control adoptees, rated blindly, had found a significantly higher prevalence of schizophrenia and schizophrenia-related disorder in the biological relatives of schizophrenic adoptees than in controls. Moreover, eight of the characteristics found in the relatives diagnosed as latent or uncertain schizophrenia were used in DSM-III to define what is designated there as schizoptyal personality disorder.

In the 9,000 adoptees outside of Copenhagen, 42 have been identified as having developed schizophrenia. These and the 42 non-schizophrenic control adoptees had 1,100 biological and adoptive relatives. Interviews have been completed on approximately 90 percent of the relatives who are alive and residing in Denmark; these interviews are presently being rated blindly and independently by two groups of raters, one group using DSM-III criteria, the other using global judgment based on the descriptions of Bleuler. The results, when the ratings are completed, will be compared with the findings in the Copenhagen study and with each other for sensitivity and specificity. In addition, a substantial number of symptoms and manifestations with possible pertinence to schizophrenia have been scored in the interviews of relatives of schizophrenic and normal adoptees. These will be used in Bayesian and cluster analyses in attempts to better characterize the syndrome of latent schizophrenia to be found according to Bleuler in the relatives of schizophrenics.

2. The Family Study in County Roscommon, Ireland

This county and others in Western Ireland are known to have a prevalence of schizophrenia which is approximately three times that found in England, Europe, and the United States. Obvious artifacts, such as selective and subjective bias, as well as idiosyncratic diagnoses have been ruled out. This observation is of great importance to an ultimate elucidation of etiologic factors in schizophrenia generally. The adoption strategy would permit an evaluation of the proportion of the higher incidence which could be attributed to genetic or family associated environmental factors but efforts to conduct such a study were unsuccessful because of the inadequacy of adoption records. A case-controlled family study, however, represents an alternative and feasible approach to distinguishing general environmental factors from specific family-related factors (genetics, diet, rearing) in accounting for the high incidence of schizophrenia in Ireland.

The Medico Social Research Board has obtained extensive information on the families of schizophrenic patients in Roscommon County, including interviews with the relatives and abstracts of hospital records. These have been made available to us and will be examined, along with similar information from families of controls, for the prevalence of schizophrenia among them. The proportion of schizophrenia in this sample, which is familial (i.e., duplicated in one or more members of the family), compared with that proportion in population samples in other countries where the prevalence of schizophrenia is not high, will be the first step in ascertaining to what extent the high prevalence in Ireland may be attributed to specific genetic and environmental factors.

Significance to Biomedical Research and to the Program of the Institute

The data gathered will shed light on the differentiation between familial and sporadic forms of schizophrenia and contribute, as well, to the illumination of genetic, as opposed to familial and environmental, factors in this disorder.

Proposed Course

We are continuing to collect data in Denmark and Ireland which will be analyzed at McLean Hospital and NIMH and prepared for publication.

Publications

Kety, S. S. The concept of schizophrenia. In M. Alpert (Ed.), Controversies in Schizophrenia. New York: Guilford Press, 1985, pp. 3-11.

Kety, S. S. Schizotypal personality disorder: An operational definition of Bleuler's latent schizophrenia? Schizophrenia Bulletin (in press).

Kety, S. S. Interactions between stress and genetic processes. In M. R. Zales (Ed.), Stress in Health and Disease. New York: Brunner/Mazel, 1985, pp. 115-129.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00672-20 LSES

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Social Psychological Correlates of Occupational Position

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. L. Kohn, Chief, Laboratory of Socio-environmental Studies, NIMH

OTHER:	C. Schooler	Research Psychologist	LSES	NIMH
	K. Miller	Research Sociologist	LSES	NIMH
	K. Slomczynski	Visiting Scientist	LSES	NIMH
	C. Schoenbach	Social Science Analyst	LSES	NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11.50	5.50	6.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The object of this study is to assess the reciprocal effects of occupational conditions and psychological functioning (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a longitudinal study in 1974, with the reinterviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children. Replications of this research have been carried out in Poland and Japan.

Project Description:

The principal goal of this research is to assess the relationships between people's job conditions and their psychological functioning. The evidence provided by this research demonstrates that job conditions have a marked impact on cognitive functioning, on values, and on conceptions of self and orientations to society. Psychological functioning, in turn, has a rather more gradual but substantial impact on job conditions.

The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One major purpose of the follow-up study has been to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators have assessed the magnitudes of the reciprocal effects of job conditions and several important facets of psychological functioning. The study of wives was designed in part to ascertain whether job conditions affect men and women similarly. The research has shown that they do. The study of the wives, and the study of the children, was also designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of children's educational and occupational experiences on their own psychological development.

During the current year, the major research efforts have been addressed to analyses of: the relationship between educational experience and children's personality development; the intergenerational transmission of values; intra-family dynamics and children's personality development; and continued cross-national comparative analyses, for Poland and Japan, of the relationships between job conditions and psychological functioning. This Annual Report summarizes all of these activities.

EDUCATIONAL EXPERIENCE AND CHILDREN'S PSYCHOLOGICAL DEVELOPMENT

The purpose of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis has been to examine the processes by which students' educational experiences, particularly the degree of self-direction they exercise in their educational endeavors, affect their psychological functioning. Data

for this analysis were collected in the 1974 follow-up survey, when one pre-selected child of each father in the sample was interviewed. The interview schedule for these "children" -- by then aged 13 to 25 -- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions, designed to parallel those earlier found to be useful for analyzing adults' occupational experience, focus on such dimensions of the educational experience as its substantive complexity and how closely it is supervised. The underlying hypothesis is that, just as occupational self-direction affects the psychological functioning of employed adults, so does educational self-direction affect the psychological functioning of students. Insofar as it is meaningful to do so, the investigators' intent has been to conceptualize and measure educational self-direction as being parallel to occupational self-direction.

Past annual reports have discussed the relationship of educational self-direction with ideational flexibily, self-directedness of orientation, and distress. The major accomplishment this year has been the development of a comprehensive model of educational self-direction and all three of these major facets of personality. The model shows an intricate system, in which educational self-direction directly or indirectly affects all three facets of personality; distress and ideational flexibility affect educational self-direction; distress affects both self-directedness of orientation and, indirectly, ideational flexibility; and self-directedness of orientation and ideational flexibility directly or indirectly affect each other. These findings have important theoretical and practical implications for our understanding of the psychological impact of education, for the understanding of work in the labor force, and for the understanding of the link between the two.

First, the findings illuminate some of the major processes by which education affects cognitive functioning. The model certainly confirms that educational self-direction affects students' cognitive functioning. The model also suggests, though, that a large part of this effect comes about through educational self-direction affecting self-directedness of orientation and distress. Thus, non-cognitive aspects of personality play a considerable role in how the school contributes to students' intellectual development. Educational self-direction leads to more effective intellectual performance as much because it leads to a self-directed orientation and because it mitigates distress as because of its role in cognitive training, per se. Dewey was right in thinking that the building of "character" is central to the educational process.

A second major conclusion is that the causal relationship between the exercise of self-direction in work and personality is remarkably similar for students and adult workers. The similarity of findings suggests some fundamental links between work, regardless of setting, and the personality of the worker. For schooling, as for paid employment, the opportunity to

exercise self-direction in one's own work has powerful psychological effects. This constitutes a striking affirmation of the applicability of an interpretative model designed to explain the social psychology of work in paid employment to the social psychology of work in school.

Finally, the research corroborates essential elements of the interpretation that schooling reproduces social class through differential training of independent, self-directed orientations in students. The investigators find, as they and other students of social stratification would expect, that students from families of higher socio-economic status are more likely to be educationally self-directed. They also find, exactly as proponents of this interpretation claim, that as students progress through the educational system, they are progressively trained to have more and more self-directed orientations. Indeed, the research goes further: it identifies a crucial element of that training. Students in higher grade levels experience more educational self-direction; this increased exercise of initiative, thought, and independent judgment in schoolwork changes the self-directedness of students' orientations, their sense of distress or well-being, and the effectiveness of their cognitive functioning. Because educational self-direction has these powerful effects, it proves to be a central mechanism through which the educational system molds the personalities of students in ways consonant with their likely positions in the hierarchical division of labor. Educational self-direction is a critical link between schooling and adult occupational role.

THE INTERGENERATIONAL TRANSMISSION OF VALUES

Melvin Kohn, Kazimierz Slomczynski, and Carrie Schoenbach's cross-national analysis of the intergenerational transmission of values addresses two principal issues: To what extent does the social stratification position of the parental family affect the values of its adolescent and young-adult offspring? What are the processes by which that position affects offsprings' values? Both questions are classic issues for the sociology and social psychology of socialization.

Although there is considerable evidence that social stratification has substantial effects on adults' -- hence, parents' -- values and orientations, there is much less evidence that the family's stratification position has appreciable effects on the values and orientations of its adolescent and young-adult offspring. Nor, for that matter, is there much evidence that parents' values have an appreciable effect on their children's values. Quite the contrary: prior research has indicated suprisingly weak relationships between parents' and children's values. And yet, as Clausen put it, "[a] basic tenet of socialization theory -- almost all socialization theory -- is that the child's core value orientations are learned in the family...."

The inquiry is premised on the belief that it is not socialization theory but past empirical evidence that is deficient. The investigators therefore hypothesized that the stratification position of the parental family appreciably affects the values of adolescent and young-adult offspring and, furthermore, that the effect is primarily through parents' values.

They further hypothesized that the relationship between social stratification and children's values, as mediated through parents' values, is not peculiar to any particular economic or political system, but is built into the structure of industrial society. Their inquiry was therefore cross-national, based on more-or-less parallel analyses of one capitalist society (the United States) and one socialist society (Poland). Obviously, even identical findings for these two countries would not ensure that the findings could be generalized to other capitalist and socialist societies. Similar findings would, however, demonstrate that the relationship between stratification and children's values is not unique to the economic and political system of either the U.S. or Poland. The intent of the inquiry was to show that the relationship between social stratification and children's values transcends the differences between capitalist and socialist societies because the same mechanisms underlie this relationship in both systems.

The further intent of this inquiry was to explicate the mechanisms by which family stratification position affects children's values. Here the investigators built on earlier analyses, which point to the pivotal role of occupational experience, particularly the opportunity to exercise self-direction in one's work. They extended those analyses in several ways: by simultaneously examining the relationship between occupational self-direction and the values of husband, wife, and child; by assessing the influence of each family member's values on the values of the others; and, basic to all the rest, by treating the family as a subsystem of the larger social system.

The analyses are based on representative samples, in the United States and Poland, of parents and their adolescent or young-adult offspring. In both countries, the samples consist of triads: father, mother, and one selected child.

The U.S. data are partially longitudinal. The baseline data consist of interviews conducted in 1964 with 3101 men, representative of all men employed in civilian occupations in the continental United States. Every man who was the father of at least one child living at home and aged 3-15 years was asked about his values vis-a-vis one of those children, randomly selected. Ten years later, in 1974, a representative subsample of one-fourth of the men in the baseline survey (687 in all) were re-interviewed. Also interviewed were the wives of men then married and

the "child" about whom the values questions had been asked ten years before -- these "children" being 13-25 years old when interviewed. There are 352 families in the U.S. sample.

The Polish data are cross-sectional. Interviews were conducted in 1978 with 1557 men, representative of all men employed in civilian occupations in the urban areas of Poland. As in the U.S. survey, each father who had at least one child aged 3-15 living at home was asked about his values vis-a-vis a randomly selected one of those children. In 1979-1980, interviews were conducted with those children who were then 13-17 years old (N=177) and with their mothers. This age-range was intended to maximize the overlap with the age-range of the U.S. "children". Although the data from mothers and children were secured one-and-a-half to two years later than those from fathers, they are treating the Polish data as if the interviews with father, mother, and child were contemporaneous.

The analyses provide evidence, for both the U.S. and Poland, not only of a close relationship between parents' and children's values, but of an actual influence of parents' values on children's values. Most past studies have underestimated the magnitudes of the correlations between parents' and children's values, because they have not taken measurement error into account and, more fundamentally, because they have not dealt with so important a dimension of values as valuation of self-direction. And, by and large, past studies stopped with correlations. The crux of the matter, however, is not the magnitudes of the correlations but the part played by parental values in the actual process of intergenerational value-transmission. The present study demonstrates that parental values have a considerable impact on offspring's values, even with many other pertinent aspects of social structure taken into account. A fundamental but contested tenet of socialization theory -- almost all socialization theory -- has hereby been confirmed.

The investigators have further found that, in both the U.S. and Poland, the stratification position of the parental family has a considerable impact on the values of its adolescent and young-adult offspring. This effect results primarily from the stratification position of the family affecting parents' values and parents' values, in turn, affecting offspring's values. Moreover, these analyses show that all the links in the causal chain are strong: Social stratification affects parental occupational self-direction; occupational self-direction affects parental values; parental values affect children's values. Whatever doubts past studies may have raised about this causal chain can now be resolved.

In both countries, parents' occupational self-direction substantially affects (and is affected by) their values. Moreover, adolescents' and young adults' educational self-direction affects their values, just as parental occupational self-direction affects parental values. This finding provides a confirmation of the larger thesis that the

experience of self-direction in one's work, whether in paid employment or in schoolwork, has a major impact on one's values. This is as true for adolescents and early adults as it is for older adults. These findings lend support to the further argument that people's own experiences become more and more important for their values, with their own social-structural positions and attendant experience eventually overshadowing the influences of their parental families.

The findings also provide an intergenerational extension of Kohn and Schooler's interpretive model for adult workers of social stratification, job conditions, and personality. In that model, social stratification affects occupational self-direction, which is reciprocally related to personality, which in turn affects job conditions and one's place in the stratification order. We can now add that social stratification affects not only adults' own values but also their children's values and their children's opportunities for educational self-direction. Parental stratification position thus affects children's values not only through the transmission of values from parents to children, but also through its effect on children's opportunities for educational self-direction.

Finally, the investigators have found one substantial difference between the U.S. and Poland: the relative roles of fathers and mothers in the intergenerational transmission of values. In the U.S., fathers play at least as large a role as do mothers; in Poland, mothers play the predominant role. This finding does not seem to be an artifact of study design. Nor does this cross-national difference seem to result from differing economic or political systems. Rather, it appears to be a cultural difference: Polish fathers play a more traditional role in the division of labor within the family and in the socialization of children. This pattern, originally characteristic of the peasantry and diffused to all segments of the society through rural-to-urban migration, is also supported by the influential Catholic church. More important than this one difference, however, is the basic similarity of findings for the U.S. and Poland: Family stratification position greatly affects adolescents' values; half or more of this effect is through parents' values; and there is a strong reciprocal relationship between occupational self-direction and values. The processes are built into the structure of industrial societies, both capitalist and socialist.

INTRA-FAMILY DYNAMICS AND CHILDREN'S PERSONALITY DEVELOPMENT

Carmi Schooler has continued working on a complementary analysis of the ways that reported child-rearing practices and family relationships affect children's psychological development. He has been using confirmatory factor analysis to develop measures of the many aspects of parent-child relationships covered by the interviews. These include parental strictness, warmth, and degree of dominance over the child, as

well as the degree to which children feel free to talk things over with each parent and the likelihood that they will turn to either parent when troubled. New measures developed this year include children's perceptions of their closeness to each of their parents, as well as indices of the communication processes that take place within the family.

During this year, Schooler has also examined the links between family behavior patterns and children's psychological functioning. These analyses not only take into account family social background, but also statistically control the parents' own psychological functioning. The findings indicate that parents who give their children the opportunity to be self-directed have intellectually flexible, self-directed children. Thus, the children of parents who tend not to control, dominate, or lay down the law to their children are more likely to have high levels of intellectual flexibility and to be self-directed in both their values and their orientations. Parents who praise their children are also more likely to have children who are intellectually flexible and who have self-directed values.

A certain amount of emotional distance from parents seems, however, to be conducive to children's intellectual flexibility and self-directedness. Parental warmth is related to lower levels of children's intellectual flexibility, as is a close relationship with the mother, as perceived by the child. A close relationship with the father is related to a conformist orientation, and a close relationship with either parent is related to conformist values. On the other hand, close parent-child relationships are positively related to children's sense of well-being. In addition, children of parents who are warm and praising and to whom the children speak freely suffer less distress. The importance of the links between close parent-child relationships, communication, and the child's sense of well-being is also attested to by the strongest finding of the analysis: a powerful relationship between family openness and interest in communication and children's sense of well-being.

Although the pattern of relationships that has emerged from these analyses seems coherent and meaningful, the nature of the underlying causal connections is uncertain (e.g., parents' praise can be either the cause or the result of children's superior intellectual flexibility). Because of this ambiguity, Schooler has devoted considerable effort this year to trying to devise linear structural-equation analyses that will permit the assessment of possibly reciprocal relationships (e.g., between children's and parents' psychological functioning; between parents' behavior and children's psychological functioning). This analysis has turned out to be exceptionally difficult. It may even prove to be impossible to definitively establish causal directionality. However, even if the nature of the causal relationships between family behavior patterns and children's psychological functioning remains ambiguous, establishing that parental childrearing practices are related to children's psychological functioning

independently of parents' psychological functioning and of social background represents an increase in our knowledge of the relationships between family life and children's psychological functioning.

THE POLISH REPLICATION

The main purpose of the Polish replication has been to see whether the interrelationship of social stratification, job conditions, and psychological functioning are similar in socialist and capitalist societies. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he and Melvin Kohn have been analyzing them. Previous Annual Reports reviewed the development of methods designed to assure cross-national comparability of indices and the analysis of two of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.? If so, do these relationships result from the greater opportunities for occupational self-direction enjoyed by men of higher social-stratification position? As reviewed in detail in earlier Annual Reports, the answers to both questions are positive with respect to values and social orientations, but not with respect to self-conception.

Further comparative analysis of the Polish and U.S. data has focused on social stratification and the intergenerational transmission of values (discussed above). Current work focuses on extending these analyses to other facets of children's personality development -- cognitive development, self-conceptions, and social orientations. The investigators have also been filling in gaps in their analyses and reassembling their findings for publication as a book, to be published in Polish by the Polish Scientific Publishers, on behalf of the Polish Academy of Sciences, who provided the Polish data and have supported the research throughout. With appropriate modifications, the book may also be published in English in the United States.

THE JAPANESE REPLICATION

Another major replication of the Kohn-Schooler occupations study has been conducted in Japan by Atsushi Naoi of Osaka University and Ken'ichi Tominaga of Tokyo University, in collaboration with Carmi Schooler. Data-collection took place during the summer and fall of 1979. At that time, a probability sample of more than 800 employed men was interviewed, using a questionnaire that asked about job conditions and aspects of

psychological functioning in ways comparable to those of the 1964 U.S. study. Data-analysis began in October, 1980, when Naoi came to the Laboratory as a Visiting Scientist to work collaboratively with Schooler. As reported in previous Annual Reports, Naoi and Schooler developed confirmatory factor-analytic measurement models of occupational self-direction, intellectual flexibility, and several facets of self-conception and social orientation. These models proved to be generally similar to those that had previously been developed for the American sample. Causal analyses of these data generally confirmed the U.S. findings.

This year, Carmi Schooler's work on the replication and extension in Japan of our U.S. research on the relationships between job conditions and psychological functioning involved both the continued analysis of previously collected data and the negotiation and planning for the acquisition of new data.

Early in the year, Schooler developed a model of the reciprocal effects of job conditions and parental valuation of self-direction. The model demonstrates that their own experience of occupational self-direction leads parents to value self-direction for their children; by contrast, ownership, high hierarchical position, and employment in a bureaucracy lead to parents valuing conformity for their children. These findings are congruent with those already reported about the determinants of self-directed orientations in Japan. The finding for occupational self-direction is entirely consonant with our U.S. findings; those for organizational position are distinctive to Japan.

The same analytic model was used to examine the relationships between Japanese men's occupational conditions and what Schooler terms their psychological integration into their social position -- their level of alienation; their identification with their social stratification position and with their social class position; and the importance they place on their jobs, their firms, and their families. In Japan, occupational self-direction reduces alienation and promotes identification with higher social classes and strata. In addition, although ownership, high hierarchical position, and employment in a bureacracy do not affect alienation, they all lead men to to identify themselves with higher social strata and higher social classes, suggesting, contrary to the beliefs of many Japanese area specialists, that socio-economic position does affect the ways Japanese identify themselves and their interests.

Another set of analyses carried out this year added an index of the relative traditionalism of Japanese industries (developed the year before by Tominaga and Schooler) to the models of the interrelationship of job conditions and psychological functioning. Although the inclusion of this index of traditionalism does not substantially change any of the previous findings, employment in a relatively traditional industry does have

psychological repercussions. Employment in such an industry leads to conformist values, less personally responsible standards of morality, and greater authoritarian-conservatism. It also results in less alienation, greater identification with higher social strata, greater self-confidence, and less self-deprecation. This pattern, in which participation in a traditional social setting leads to a sense of social integration, to acceptance and identification with authority, and to comfort with oneself is congruent with the interpretations of a school of social theorists that traces back to Durkheim.

Given the richness of the comparative findings, it seems highly desirable that the parallel analyses of American and Japanese data be extended. To that end, Schooler is working intensively on an attempt to replicate in Japan, with the limited resources available, the full set of studies on the interrelationship of occupational and familial conditions and psychological functioning that we have carried out in the United States. Plans are currently under way for our Japanese collaborators to re-interview the original sample of employed men, to obtain longitudinal data parallel to those we have for American men. The wives of the original respondents have already been interviewed by the Japanese investigators. Schooler is presently conducting negotiations to make these data available for comparative analyses of the psychological effects of housework and paid employment on women in Japan and the United States -- countries in which the social position of women is quite different. Finally, plans have been initiated for a jointly-sponsored effort to parallel in Japan our research on the intergenerational transmission of values and modes of psychological functioning. This is to be done by interviewing the child who was the focus of the childrearing questions asked in the men's interview. The replication of this aspect of the American study is seen as particularly important because there is evidence suggesting marked differences between generations in Japan; a great deal might be learned by a comparative examination of the nature of psychological continuity in Japanese and American families.

Significance of the research:

This research is significant to the mission of the Institute on three distinct levels: (1) It has been well established that the incidence of schizophrenia is inversely related to social-stratification position. This relationship is not simply a function of greater genetic vulnerability of people at lower stratification levels or of more stressful life conditions at those levels. In larger part, it seems to result from people at lower stratification levels having less effective psychological mechanisms for coping with stress and uncertainty. This research, at a basic level, is investigating what there is about the conditions of life associated with social-stratification position that results in people of lower social-stratification position having less effective mechanisms for

coping with stress and uncertainty. (2) Above and beyond NIMH's interest in mental disorder, per se, the Institute has a mandate to study the conditions that facilitate and those that interfere with effective psychological functioning. This research has demonstrated that job conditions have appreciable effects on cognitive functioning, values, self-conceptions, and orientations to the outside world. Much of the work in this research project has focused on (a) demonstrating that job conditions actually do have a causal impact on effectiveness of psychological functioning and (b) elucidating the processes by which job affects psychological functioning. (3) As the research focuses more and more on the effects of social structure on the personality development of children, the potential relevance of what is learned for programs of prevention of mental disorder increases all the more, if only because some of the causal variables are particularly amenable to planned intervention.

Proposed course of further research:

The analysis of the processes by which parents' values and practices affect the values and personality development of their children requires much further analysis, both with respect to extending the analysis of the intergenerational transmission of values to include other facets of children's personality development, and with respect to intrafamily dynamics. Moreover, as is evident above, the analysis of the Japanese replication is incomplete, with much more to be done. Our Japanese collaborators have also collected further data, and have plans for new data-collection, which will permit a considerable enlargement of the base for comparative analysis. In particular, these data should make possible comparative analyses of the relationship between women's job conditions and psychological functioning, as well as comparative analyses of the psychological development of children.

Publications:

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Kohn, M.L., and Schooler, C.: Praca A Osobowosc: Studium Wspokzaleznosci. (Work and Personality: Study of their Interrelationships.) Warsaw: Polish Scientific Publishers. (In press)

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Wolfgang (Eds.): Arbeitsbiographie und Personlichkeitsentwicklung. Germany: Verlag Hans Huber. (In press)

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Naoi, A., and Schooler, C.: Occupational conditions and psychological functioning in Japan. Am. J. Sociol., 90: 729-752, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00679-05 LSES

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Equation Models in the Analysis of Data with Measurement Error

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ronald J. Schoenberg, Research Sociologist, LSES, NIMH

OTHER: C. Schooler Research Psychologist LSES NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	1.50	0.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this work is to further develop the methods and techniques for the specification and estimation of the parameters of structural equation models of survey data that contain random and nonrandom measurement error. Included in this are methods for the identification of the models, estimation of the means of unobserved variables, the determination of model condition, and the treatment of polytomous variables.

Project Description:

This year Ronald Schoenberg and Carmi Schooler have continued their investigation of the hypothesis, current in the fields of sociology and economics, that there are two principal sectors in the United States economy, a primary sector and a secondary sector. Proponents of this position maintain that the sector in which people work affects their economic compensation and occupational conditions. If so, it seems likely that the sector in which people work would also affect their psychological functioning.

To explore the consequences of economic sector for the psychological functioning of workers, Schoenberg and Schooler have developed an approach to its definition that differs somewhat from previous approaches, one that reflects more completely the idea that the sectors may differ in their underlying structure. Their approach does not assume in advance how many sectors there might be.

Variance in underlying structure implies that the distributions of industry measures in each sector will have different moments, that is, the means, variances, and covariances will be different in each sector. By applying a statistical method called mixture analysis to the industry measures, it is possible to estimate the means, variances, and covariances for each sector without assuming in advance which industry belongs in which sector. Thus, sector membership of each industry is an additional parameter that is estimated in the model. Provided there are enough industries in a given sector, it should be possible to use the estimated sector variance-covariance matrix to estimate parameters of a structural model describing the interrelationships of the industry variables and their relationship with workers' psychological functioning. If this is done for each sector, it should then be possible to determine the precise effect of sector on workers' psychological functioning.

To carry out this analysis, Schoenberg and Schooler secured two data-sets that purport to contain the necessary industry data. Their analyses quickly revealed that one of these data-sets was riddled with inaccuracies. The mixture analysis of the second data-set has now been completed. Schoenberg and Schooler have found not two, but at least six, sectors in the U.S. industrial economy. Seven-, eight-, and nine-sector models fit the data significantly better than does a six-sector model, but this appears to capitalize on chance, since the three additional sectors do not add substantively to the model.

Whatever the number of sectors, two sectors consistently emerge from what had previously been considered to be the primary sector. One of them contains industries that tend to have a high proportion of government sales (ordnance, aircraft and ships, communication, industrial chemicals) and the other contains other important large-scale industries (steel, machinery, computers, household appliances, photographic equipment, textiles, plastics, footwear). These two sectors rank first and second, respectively, in size, profit, and concentration. The second sector ranks first in growth, autonomy, assets, employee benefits, and unionization, and outranks the first in productivity and employee salaries. Other sectors seem to center on raw material processing and refining; retail sales; wholesale sales; printing, commercial research and general contracting. In evaluating the apparent meaningfulness and cohesiveness of these sectors, it should be borne in mind that the sectoral distinctions are not based on similarity of product but on the similarity of such industry characteristics as average number of workers, amount of assets per company, and degree of concentration.

Significance of the Research:

The determination that there are sectors in the U.S. economy has implications for the analysis of models of job conditions and worker psychological functioning in general. It suggests that anyone doing research in these areas will have to take into account the sector location of the workers and the firms that employ them. These findings will also be an important contribution to the growing literature on the definition of industrial sectors.

The mixture analysis developed for the problem of defining industrial sectors belongs in a more general class of methods for the analysis of latent variables. Work in this and related areas will have implications for research on schizophrenia and other mental disorders. Mixture analysis, for example, has some promise for detecting and measuring the latent dimensions of schizophrenia.

Proposed Course of Further Research:

In the next step in the research on industrial sectors Schoenberg and Schooler will look at models relating company and worker characteristics within sectors. Measures of psychological functioning from the data set collected by the laboratory will be aggregated by industry and incorporated into

the model. They will then be in a position to determine the consequences of sector position on the relationships among company and worker variables.

In addition, the methods developed for categorical and mixture analysis will be extended to applications in medical diagnosis, in particular the diagnosis of schizophrenia. These methods should be able to help solve very serious problems in determining the variety of types of schizophrenia as well as help in the development of techniques for the diagnosis of schizophrenia in particular cases.

Publications:

Schoenberg, R.J.: Using LISREL to Improve Prediction with Multicollinear Predictors. Richard Cook (Ed.): Proceedings of the ISSUE84 Conference, SPSS, Inc., Chicago, 1984, pp. 67-84.

Schoenberg, R.J. and C. Richtand: An Application of the EM method to the Maximum Likelihood Estimation of Multiple Indicator and Factor Analysis Models. Sociol. Res. Methods, 13: 127-150, 1984.

Schoenberg, R.J.: Statistical methods must be appropriate: A reply to Pat McGowan. Review, 8: 1985, 513-515.

Schoenberg, R.J.: Latent Variables in the Analysis of Limited Dependent Variables. In Nancy Brandon, (Ed.): Sociological Methodology, California, Jossey-Bass, 1985, pp. 212-241.

Schoenberg, R.J.: Statistical Issues for Research on Social Drinkers. In Marc Galanter (Ed.): Recent Developments in Alcoholism. Vol. III. New York: Plenum, 1985, pp. 289-300.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00680-03-LSES

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Work Experiences and the Deinstitutionalized Mentally Ill

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Elliot Liebow, Guest Researcher, LSES, NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this exploratory, participant observation study is to examine the work experience of the deinstitutionalized mentally ill over time and to seek out ways in which job characteristics, symptoms, and social relationships interact with one another to effect the course of recovery from psychiatric disorder and reintegration into the community. Field work was carried out with residents of halfway houses, participants in community-based psychosocial and transitional work programs, and with "unattached" deinstitutionalized men and women.

Project Description:

Two years ago, Elliot Liebow, on detail to the Laboratory from the Extramural Program, began an exploratory, participant-observer study of the relationship between work experience and recovery from mental illness. The goal of this exploratory research was not to test hypotheses but rather to grasp, so far as possible, the dynamics of the interaction between work experiences and recovery from mental illness.

Data collection was based mainly on direct observation and personal interaction with men and women coming out of Springfield State Hospital and following them through their reentry into their home communities, focussing particularly on the role of work experiences in this process. The study population was drawn mainly from half-way houses and psychosocial and vocational programs in Montgomery County, Md.

Last year, while still collecting data, Liebow was stricken by two successive major illnesses. He retired on disability last September but remained as a guest researcher in order to try to salvage some of the data he had already collected. These were somewhat too thin to serve their original purposes but were potentially useful nonetheless.

In November, Liebow began collecting data as a participant observer in a shelter for homeless women in Rockville. Most of the two dozen women who are "regulars" (in the sense that they stay at the shelter night after night, month after month) as well as the more casual users who come for a night or two, have a history of mental illness and/or institutionalization. Liebow's plan now is to do a participant-observer study that contrasts the dynamics and outcomes of two post-institutionalization life-styles: the highly structured, tightly supervised group living of halfway houses and psycho-social day programs (data collected last year) versus the relatively unstructured, free floating life style of shelters and soup kitchens (data currently being collected).

The principal focus will be on the two dozen women who are "regulars" at the shelter. Liebow has now followed them intensely for more than seven months and expects to continue this data collection, including life histories, across the four seasons, ending in November of this year. He is confident that the contrasting data on halfway house residents, though somewhat intermittent and less systematic, will nevertheless be sufficiently rich and deep so as to make a useful and suggestive comparison of the two major paths taken by persons coming out of institutions.

Significance of the Research:

This project is directly pertinent to our understanding of rehabilitation of the deinstitutionalized mentally ill.

Proposed Course of Further Research:

Formal data collection has been completed. The investigator has retired from Government employment under disability, following serious illness. He continues to work on these and other data as a Guest Researcher, but intermittently.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00424-10 LCB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biologically Active Peptides in the Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael J. Brownstein, Chief, Laboratory of Cell Biology, NIMH

(see attached)

COOPERATING UNITS *(if any)* LMMB/NCI; St. Louis U. Med. Ctr.; LDN/NICHHD; Bowling Green State Univ.; NIAAA; FDA; USUHS; INSERM; LMG/NINCDS; LCM/NIMH; LMG/NICHHD; LNP/NINCDS; Genentech, Inc.; Univ. Victoria; Univ. New Hampshire; LMB/NINCDS; Univ. of MD

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10		0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued to study the distribution of peptide-containing cells in the central nervous system, the biosynthesis of biologically active peptides, and the factors that regulate peptide secretion. Our studies of a number of peptides have contributed to a better understanding of the cell biology of peptidergic neurons and of their role in the brain.

Other Professional Personnel Engaged on Project

H.-U. Affolter	Visiting Fellow	LCB, NIMH
F. Antoni	Visiting Associate	LCB, NIMH
J. Barbet	Guest Researcher	LMMB, NCI
M. Beinfeld	Assoc. Professor	St. Louis U.Med.Ctr.
T. Bonner	Res. Biophysicist	LCB, NIMH
M. Brann	Guest Researcher	LCB, NIMH
D. Brenneman	Sr. Staff Fellow	LDN, NICHHD
R. Conner	Professor	Bowling Green SU
J. Dave	Visiting Scientist	NIAAA
H. DeVoe	Assoc. Professor	Univ. MD
M. Dohadwala	Visitng Fellow	LMB, NINCDS
L. Eiden	Sr. Staff Fellow	LCB, NIMH
B. Fraser	Lab Chief	FDA
D. Forman	Asst. Professor	USUHS
P. Giraud	Research Sci.	INSERM
V. Hook	Guest Researcher	LCB, NIMH
C.-M. Hsu	Phys. Sci. Tech.	LCB, NIMH
A. Iacangelo	Microbiologist	LCB, NIMH
C. Jelsema	Guest Researcher	LCB, NIMH
D. Kligman	Staff Fellow	LCB, NIMH
K. Koller	Guest Researcher	LCB, NIMH
R. Lazzarini	Lab Chief	LMG, NINCDS
D. Marshak	Guest Researcher	LCB, NIMH
R. Martenson	Res. Chemist	LCM, NIMH
E. Mezey	Visiting Associate	LCB, NIMH
J. Moskal	Sr. Staff Fellow	LCB, NIMH
H. Okayama	Visiting Scientist	LMG, NICHHD
M. Palkovits	Visiting Scientist	LCB, NIMH
R. Pruss	Sr. Staff Fellow	LCB, NIMH
U. Rapp	Section Chief	NCI
T. Reisine	Sr. Staff Fellow	LCB, NIMH
A. Rokaeus	Visiting Fellow	LCB, NIMH
G. Rougon	Visiting Fellow	LCB, NIMH
J. Sarvey	Assoc. Professor	USUHS
A. Schaffner	Sr. Staff Fellow	LNP, NINCDS
P. Seeburg	Section Head	Genentech, Inc.
E. Shepard	Microbiologist	LCB, NIMH
N. Sherwood	Prof. Asst.	Univ. Victoria
R. Siegel	Staff Fellow	LCB, NIMH
S. Sower	Asst. Professor	Univ. NH
P. Stanton	Res. Assoc.	USUHS
A. Stone	Res. Chemist	LCB, NIMH
J. Waschek	Guest Researcher	LCB, NIMH
W.S. Young	Guest Researcher	LCB, NIMH
R.T. Zoeller	Guest Researcher	LCB, NIMH

Stated in its most general form, our goal is to understand the development and function of the nervous system. In particular, we are attempting to isolate, characterize, and study molecules with important roles in the brain and periphery. Examples include peptide neurotransmitters, receptors, factors that regulate neuronal development, and proteins involved in the uptake, storage and biosynthesis of neurotransmitters. The first step in this process involves developing assays for molecules of

interest and using the assays to detect these molecules in the course of their purification. In this way we have succeeded in isolating a gonadotropin releasing factor from lamprey, a neurite extension factor, and a neuronal cell adhesion molecule. Antibodies against the purified factors (or against impure preparations) can be prepared and used for anatomical, biochemical, cell biological and molecular biological studies; or the purified proteins can be subjected to chemical analyses. Even the partial amino acid sequence of a peptide or protein can be very useful to the molecular biologist for isolating DNA complementary to a particular messenger RNA species. In the last year we have exploited such sequence information to detect a number of cDNA's including those for chromagranin, galanine, the raf gene product, and substance P. The cDNA's can be sequenced and the structure of the proteins that they encode inferred. Furthermore, the cDNA's can be employed to isolate their corresponding genes and as probes for studies of messenger RNA production. A particularly exciting method based on the use of small synthetic cDNA probes has been perfected: in situ hybridization cytochemistry. This technique allows one to visualize (and, in principle, to quantitate) mRNA levels in individual nerve cells on brain sections. It should be very useful for studies of central nervous system development and metabolism.

Project Description

Peptide/protein isolation and characterization

Drs. Marshak, Fraser, Sherwood, and Sower have purified the gonadotropin releasing hormone from lamprey, determined its amino acid sequence, and synthesized the peptide. Their work may contribute to a better understanding of lamprey reproduction and allow a method for eradicating this fish predator from the Great Lakes to be developed.

Drs. Kligman and Marshak have isolated a neurite extension factor from the bovine telencephalon and have nearly completed their characterization of it.

Dr. Rougon has purified the rat and bovine neuronal cell adhesion molecules (N-CAM); she and Dr. Marshak have sequenced the N-termini of these proteins, synthesized a peptide corresponding to the N-terminus of the bovine molecule, and raised an antibody against this peptide which recognize the native N-CAM molecule.

Cloning and sequencing of cDNA's and genomic DNA's

Dr. Bonner has continued a collaboration with Dr. Rapp of NCI to characterize the raf oncogene. The gene encodes a serine-threonine specific protein kinase which is expressed in a variety

of tissues including brain. They have deduced the complete amino acid sequence of the human protein from a nearly complete cDNA and have expressed the protein in E. coli. They have proposed that the protein is a protease-activated kinase which mediates growth factor signals and are currently testing this hypothesis. They have also used the cDNA to identify all but the first exon in the cloned human gene and have identified other cDNA's which encode a related protein with a different tissue distribution.

Dr. Bonner has cloned the complete rat substance P gene and most of the human gene and has mapped it to human chromosome 7. Drs. Affolter, Bonner and Lazzarini have cloned a nearly complete human substance P cDNA. Sequence comparisons of the rat, human and bovine genes indicate: (a) that the amino acid sequence of the entire precursor protein is highly conserved, suggesting a function for the portions not encoding substances P and K, and (b) that there is a very extensive region (200 nucleotides) of conserved (80%) sequence in the promoter region which suggests an unusual complexity to the regulation of the gene. Dr. Bonner has recently cloned a second human tachykinin gene which encodes neuromedin K (neurokinin β) and is in the process of characterizing it.

A member of a family of acidic, secreted glycoproteins, chromogranin A is a 72000 dalton protein which is the main secretory protein of the adrenal medulla and is also present in parathyroid, pituitary, brain, enteric nervous system, endocrine pancreas, thyroid, thymus, retina and natural killer cells of the immune system. Wilson et al., have shown in fact that chromogranin is contained in chromophobe cell of the anterior pituitary, making chromogranin the first identified secretory product of these cells. In order to understand what the function of this ubiquitous protein might be, and to characterize it as a marker of the diffuse neuroendocrine system Eiden and Iacangelo have cloned and are characterizing the bovine cDNA and gene for chromogranin A. To begin to understand the function that chromogranin A might play in endocrine cells, Chang-Mei Hsu has transfected Ms. Iacangelo's chromogranin A cDNA clone into rat fibroblasts and isolated several cell lines that have integrated the chromogranin processed gene and stably express chromogranin messenger RNA. It is hoped that a careful examination of the phenotype of these cells will afford some clues as to the function of chromogranin A in the endocrine system. Hsu and Iacangelo are also studying the regulation of chromogranin A biosynthesis in cultured chromaffin cells. Interestingly, elevated potassium and veratridine, which strongly induce enkephalin mRNA in these cells (see below), do not seem to affect the expression of the chromogranin message, indicating that the two major secreted proteins of the adrenal medulla are not under coordinate regulation in these cells.

Dr. Rokaeus has cloned and characterized cDNA for porcine galanine. Dr. Koller has made good progress toward cloning the cDNA for peptide VQY.

Drs. Brownstein and Okayama are in the process of developing a novel bacterial expression cloning system that should allow fast and simple selection of positive clones with antibodies.

Drs. Siegel, Brownstein, and Okayama have constructed several very large cDNA libraries from neural and endocrine tissues and have begun their attempts at expression cloning in mammalian cell lines.

Use of cDNA's and cRNA's for in situ hybridization cytochemistry

Drs. Siegel and Young have succeeded in developing a fast and reliable technique for visualizing specific mRNA's in neurons on brain sections and cells in tissue culture. It should prove possible to convert this from a qualitative to a quantitative method. Drs. Siegel and Young have used the method to visualize proopiomelanocortin-, enkephalin-, vasopressin-, oxytocin-, CRF-, and CCK-mRNA's to date. Drs. Young, Mezey, and Siegel have been able to combine the immunocytochemical and *in situ* hybridization methods, allowing them to see both peptide hormones and mRNA's in the same cell. Dr. Zoeller has begun to examine the distribution of cells that manufacture GnRH mRNA.

Light and EM immunocytochemistry

Dr. Mezey has continued to study the coexistence of peptides in the hypothalamic paraventricular nucleus (PVN). She has detected VIP positive neurons in the PVN after adrenalectomy and during lactation. In addition, she succeeded in demonstrating the coexistence of CCK and vasopressin in cells that contain corticotropin releasing factor (CRF). The former two peptides, given together, stimulate ACTH release from primary cultures of pituitary as well as CRF does. Using electron microscopic immunocytochemistry Dr. Mezey has visualized CCK and vasopressin in the same secretory granules in the median eminence.

Dr. Mezey has detected the glycopeptide portion of the vasopressin precursor in the vasopressin deficient Brattleboro rat. Furthermore, she has found that adrenalectomy results in an increase in urine osmolarity in these rats, suggesting that they may have a limited capacity for vasopressin biosynthesis.

Drs. Mezey and Seeburg have mapped the distribution of the recently discovered GnRH associated peptide (a prolactin release inhibiting protein itself) in the brain of the Rhesus monkey, and shown that it coexists with GnRH.

Drs. Forman (Dept. of Anatomy, USUHS), Mezey, and Pruss have

demonstrated that both Neuropeptide Y and enkephalin are stored in the same secretory granules: Drs. Pruss and Forman developed methods for using video intensified immunofluorescence microscopy to identify colocalized neuropeptides in purified chromaffin granules, and Dr. Mezey used double-label immunoelectron microscopy as a second method for demonstrating peptide colocalization within cells of the bovine adrenal medulla. The identification of these two peptides in the same secretory granule is of interest since they are not synthesized on a common precursor, and appear to be independently regulated in chromaffin cells.

Dr. Pruss has also collaborated with Dr. Rokaeus in localizing the peptide galanin in adrenal medulla and in chromaffin cell culture where it coexists with enkephalin.

Studies of receptors

Dr. Antoni has examined the vasopressin receptors in several tissues in detail. He has shown that the receptor in the anterior pituitary is pharmacologically unique.

Dr. Brann has attempted to purify and characterize the dopamine receptor and has had some success in solubilizing this receptor and in developing useful affinity columns. He and Dr. Jelsema have explored the interactions between the DA receptor and other membrane proteins.

Studies of peptide and protein production, secretion, and function

Drs. Rougon, Reisine and Jacques Barbet have developed a method for introducing a heat-stable protein kinase inhibitor into cultured cells. They have used antibody targeting of protein A-coupled liposomes loaded with the protein kinase inhibitor. Dr. Rougon has found that the ACTH-secreting mouse pituitary cell line, At-T20, express the embryonic form of N-CAM. Using anti-N-CAM and the loaded liposomes, they have been able to prevent the cAMP induced secretion of ACTH from these cells, while phorbol ester and potassium stimulated release are unaffected. Along with Dr. Urs Affolter, Drs. Rougon, Barbet, and Reisine have shown that when the targeted liposomes deliver the protein kinase inhibitor to the cells, cAMP stimulated ACTH mRNA synthesis is also blocked.

Because primary chromaffin cell cultures contain multiple subpopulations of chromaffin cells, Drs. Pruss, Waschek and Eiden have screened a number of neuroblastoma cell lines for cAMP, phorbol ester, or depolarization inducible VIP. The mouse N18 cell line, the neuroblastoma glioma hybrid, NG108, and two out of three human neuroblastoma cell lines have inducible VIP. Both human cell lines also make cytochrome b_{561}. Dr. Eiden has

obtained a cDNA probe for the human VIP prohormone. Drs. Waschek, Eiden and Pruss are studying the regulation of VIP mRNA and peptide production in the human neuroblastoma cell lines in response to cAMP, phorbol ester, and depolarization. Despite strong synergism between cAMP elevation and phorbol ester treatment on both VIP mRNA and peptide levels, cAMP alone has little or no effect on either VIP mRNA or VIP peptide synthesis. Since these cell lines also make cytochrome b_{561}, Dr. Pruss will determine whether a secretory vesicle fraction exists in the human cell lines, and determine whether secretory vesicle synthesis is regulated by factors which induce VIP synthesis in these cell lines.

Dr. Pruss has continued to work on identifying cell culture conditions and intracellular pathways which regulate neuropeptide expression. She has focused on vasoactive intestinal polypeptide (VIP), a peptide which is expressed only at low levels if at all in bovine adrenal medulla. She has compared regulatory signals for VIP with those for enkephalin. Cell density plays an important role in regulating the appearance of VIP (high densities enhance the appearance of VIP) while high density lowers the amount of enkephalin in the cell cultures. Although VIP and enkephalin are both increased by nicotine stimulation, depolarization by elevated potassium or veratridine and by elevated intracellular cAMP, Drs. Pruss, Eiden, Moskal and Beinfeld (Dept. of Pharmacology, St. Louis Univ. Med. School) have recently shown that VIP is elevated independently of enkephalin levels by phorbol esters (activators of intracellular protein kinase C). The effect of phorbol ester on VIP levels is additive with that of cAMP.

Dr. Waschek has been examining the roles of intracellular cyclic AMP and calcium in regulation of the expression of vasoactive intestinal polypeptide and enkephalin in chromaffin cells in primary culture and, in collaboration with Dr. Pruss, in human neuroblastoma cell in culture. In collaboration with Dr. Jitendra Dave of the NIAAA, it has been shown that nicotine, which stimulates the release of enkephalin peptides and catecholamines from the adrenal medulla, also directly stimulates adenylate cyclase in chromaffin cell membranes, and that elevated cyclic AMP causes an elevation of enkephalin mRNA and enkephalin peptide levels. Dr. Urs Affolter demonstrated that the increase in enkephalin mRNA caused by both nicotine and elevated cAMP was preceded by an increase in the nuclear RNA precursor for preproenkephalin mRNA, suggesting that transsynaptic induction of enkephalin peptide production in the adrenal medulla is mediated by a direct activation of the enkephalin gene. Drs. Eiden, Affolter, and Giraud have demonstrated that nicotinic activation of both enkephalin release and elevation of enkephalin mRNA is dependent on calcium influx, and have termed this mutual dependence of secretory hormone release and biosynthesis on calcium influx "stimulus-secretion-synthesis coupling" after the

nomenclature of Douglas and Rubin who first described calcium-dependent release of catecholamines from adrenomedullary tissue in response to nicotinic stimulation. Dr. Ruth Siegel has demonstrated that cell depolarization with elevated potassium also strongly stimulated enkephalin biosynthesis in cultured chromaffin cells. Elevated potassium also causes an increase in enkephalin mRNA which is completely dependent upon calcium influx. Dr. Waschek has since examined the effects of barium, which facilitates calcium entry into chromaffin cells, on enkephalin biosynthesis in chromaffin cells and shown that barium causes a calcium-independent, but D600-sensitive release of enkephalins and a calcium-dependent, D600-insensitive elevation of enkephalin mRNA under the same conditions of stimulation. He tentatively concludes, and is in the process of proving, that while secretion and biosynthesis of secreted neuropeptides are coupled by a dependence on calcium influx, these processes are activated by cation influx through two different sets of channels, one activated by barium and sensitive to blockade by D600 and the other activated by calcium or barium and insensitive to D600.

Drs. Hook and Pruss have studied the subcellular distribution of neuropeptide processing enzymes as well as the regulation of these enzymes' levels and specific activities in chromaffin cells which have been stimulated to synthesize or store more neuropeptides.

Reserpine treatment of chromaffin cells increases carboxypeptidase activity and (Met)enkephalin levels are also elevated. The carboxypeptidase exhibited a higher affinity for its substrate (K_m). Further, the number of enzyme molecules (measured by RIA) remained constant. This suggests that less active enzyme molecules may have been converted to more active molecules displaying a higher affinity for substrate. Indeed, populations of carboxypeptidase enzyme at high and low states of activation have been found in different subcellular fractions. In contrast to reserpine, forskolin had no effect on carboxypeptidase activity although (Met)enkephalin levels were also increased. Other studies by Dr. Eiden have shown that while reserpine enhances processing of existing enkephalin precursors, forskolin induces synthesis of new enkephalin precursor through elevated enkephalin mRNA. Processing enzymes and/or mRNA for peptide hormone precursors may represent two different points of control in the regulation of peptide hormone production.

Dr. Pruss has raised a monoclonal antibody to cytochrome b_{561}. She and Ms. Shepard have been mapping the distribution of this cytochrome in bovine and primate neural and endocrine tissue. The distribution is being compared with that of other chromaffin granule components: neuropeptides, dopamine β-hydroxylase (DBH), chromogranin A, and to a carboxypeptidase processing enzyme (using antibodies to the purified enzyme

prepared in collaboration with Dr. Hook). The identification of cytochrome b_{561} in peptidergic cells of the retina, enteric nervous system and in all three lobes of the pituitary have provided evidence that this cytochrome may be required by peptide processing enzymes as well as the catecholamine biosynthetic enzyme, DBH.

Physiological studies of the role of VIP in the developing nervous system are being carried out by Drs. Eiden and Siegel in collaboration with Dr. Doug Brenneman of the NICHHD. These investigators have shown, using mouse spinal cord cells in primary culture, that the ontogeny of VIP expression in the spinal cord is paralleled by the tetrodotoxin-sensitive release of VIP into the culture medium of these cells, and the ability of exogenous VIP to reverse the developmentally-specific neurotoxic effects of tetrodotoxin applied to these cells. Since one action of tetrodotoxin is to inhibit the release of endogenous VIP into the culture medium, these results suggest that VIP may be an endogenous neuronal growth/survival factor in spinal cord. Dr. Brenneman has shown that glial cells cultured from developing spinal cord elaborate and release into the medium (but only in the presence of VIP) a factor which does indeed enhance the survival of spinal cord neurons.

Drs. Moskal and Pruss have examined the effect of gangliosides on neuropeptide expression in primary chromaffin cell cultures. Since gangliosides may be involved in a number of cell surface recognition phenomena, Drs. Pruss and Moskal will determine if exogenously added gangliosides can mimic the cell density effects on neuropeptide expression. In collaboration with Dr. Eiden, they have already shown that bovine brain gangliosides can specifically elevate enkephalin peptide levels as well as enkephalin mRNA. They will determine whether the ganglioside mediating this effect is specific to brain and try to identify the specific ganglioside involved.

Drs. Moskal, Schaffner, and Rougon have found that a specific, blood group-related glycolipid galactosyltransferase activity is stimultated by dibutyrl-cAMP in the synapse-competent cell line NG108-15. When NG108-15 cells are cocultured with fetal rat muscle, under conditions that induce synapse formation, this enzyme activity is also stimulated. Studies with a monoclonal antibody directed against the neuronal cell surface adhesion molecule, N-CAM, have shown that dibutyrl-cAMP inhibits the expression of N-CAM whereas coculture--as above--stimulates its expression. Muscle conditioned medium and muscle exudate have been shown to stimulate galactosyltransferase activity, whereas this enzyme is inhibited as cultures of NG108-15 cells approach confluency. These studies further support the idea that specific enzymes involved in glycosylation play a key role in synapse formation.

Dr. Moskal has identified several monoclonal antibodies that bind to cell surface proteins found highly restricted to the hippocampal formation in the adult rat. One antibody, G6E3, has been used successfully in concert with a fluorescence activated cell sorter to isolate and maintain in culture hippocampal neurons (in collaboration with A. Schaffner). This antibody also cross reacts with murine neurons and may be useful in neurological mutant studies.

A second antibody, B6E11, has also been found to be highly specific for hippocampal neurons. It recognizes cell surface protein that is developmentally regulated and changes molecular weight during early postnatal development. Drs. Moskal, Stanton and Sarvey, Dept. of Pharmacology, USUHS, have found that this antibody can block the formation and maintenance of long term potentiation (LTP) in hippocampal slices. They have also been able to show that, in the pyramidal layer of the hippocampus, the antibody is an effective blocker only at dendritic sites of potentiated neurons. When the antibody is ejected into the cell body layer no LTP blockade was observed.

In collaboration with Dr. R. Conner (Bowling Green State University), it was found that affinity-purified B6E11 injected intrahippocampally into neonatal rats was able to significantly elevate activated (REM) sleep.

Dr. Stone has utilized low uv CD spectroscopy to determine a number of disaccharide sequences of a multifunctional octadecasaccharide of the heparin (H)-heparan sulfate (HS) class of proteoglycans. This enabled a proposed structure containing two similar functional oligosaccharide (OligoS) units and the speculation of the general significance of such units. Drs. Stone and DeVoe have shown by their method of experimental and theoretical extrinsic CD spectroscopy that these OligoS may also have special binding properties for cationic aromatic ligands.

Drs. Stone and Dohadwala have found that GAG and OligoS render the plasma membranes of up to 50 percent of dissociated adrenal chromaffin cells permeable to erythrosin B (a 'small-molecule' probe), apparently nonspecifically due to their polyanionic character.

Drs. Stone and Martenson have determined the conformations of various sequential peptides of myelin basic protein (MBP) in aqueous trifluoroethanol. The finding of a sizeable degree of β-structure that is both stable in one portion and undergoes β-structure$\rightarrow\alpha$-helix transitions in other portions of the protein is significant in understanding the manner of dimerization and aggregation of MBP during compaction of the myelin sheath around CNS neurons.

Significance to Biomedical Research

Nerve cells use chemical "transmitters" to communicate with one another and with other target cells. Changes in transmitter biosynthesis, release, and/or metabolism have been suggested to result in nervous and mental disorders. Death of dopaminergic neurons in the substantia nigra, for example, is associated with the symptoms of Parkinson's disease. In the last ten years the number of putative neurotransmitters has increased by a factor of four or five. Most of the newly detected chemical messengers are peptides. Our knowledge of the anatomy, physiology and pharmacology of peptidergic neurons is comparatively incomplete at present; indeed, it is clear that many biologically active peptides remain to be isolated and characterized. The work outlined above is principally devoted to improving our understanding of cells. To the extent that we understand these cells, we can formulate better hypotheses about their role in causing disease.

Proposed Course

The work outlined above is still in progress and will be continued.

Publications

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Zamir, N., Zamir, D., Eiden, L.E., Palkovits, M., Brownstein, M.J., Eskay, R.L., Weber, E., Faden, A.I., and Feuerstein, G.: Methionine and leucine enkephalin in rat neurohypophysis: Different responses to osmotic stimuli and T_2 toxin. Science 228: 606-608, 1985.

Stone, A.L.: Far ultraviolet circular dichroism and uronic acid components of anticoagulant deca-dodeca-tetradeca- and octadecasaccharide heparin fractions. Arch. Biochem. Biophys. 236: 242-253, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00422-14 LCB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropharmacology of Circadian Rhythms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Zatz	Section Chief	SBP, LCB, NIMH
Others:	J. Moskal	Sr. Staff Fellow	LCB, NIMH
	J. Wallingford	Guest Researcher	LCB, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Section on Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Circadian rhythms and environmental lighting regulate a number of endocrine and behavioral functions. Chick pineal cells remain rhythmic and responsive to light in culture. Light, membrane potential, and norepinephrine regulate melatonin secretion and might also effect vitamin A metabolism.

Project Description

Objectives: To elucidate the biochemical mechanisms and neuropharmacology of circadian rhythms; to elucidate the effects of light and circadian rhythms on retinoid metabolism.

Methods: Biochemical, pharmacologic, cell culture, and radioactive trace techniques.

Major Findings: A system for the study (and assay) of [^{3}H]-melatonin secretion by dispersed chick pineal cells in static primary culture (for several weeks) has been developed. These cells maintain a circadian rhythm of melatonin secretion for several days and a driven rhythm for several weeks. They can be driven by light-dark cycles, alternating high and low potassium concentrations, or alternating presence and absence of norepinephrine. Norepinephrine, low potassium, and light have similar effects.

The cultured chick pineal cells are photosensitive and the action spectrum suggests mediation by a rhodopsin-like molecule, which contains retinaldehyde. The cells take up [^{3}H]-retinol and synthesize [^{3}H]-retinaldehyde and [^{3}H]-retinyl palmitate. Preliminary data suggest that retinaldehyde is also secreted into the medium.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, etc. and are primarily regulated by light-dark cycles. The mechanisms generating and regulating circadian rhythms are of broad clinical and biologic interest. Vitamin A metabolism is a critical component of visual function and is primarily regulated by light and circadian rhythms. A photo-sensitive cultured cell system (with a biochemically measurable output) has unique advantages for the investigation of the biochemical mechanisms regulating vitamin A functions.

Proposed Course of Project: The relationship between membrane potential, norepinephrine, cyclic nucleotides, light, and melatonin synthesis will be explored. The effects of light and circadian rhythms on retinoid metabolism and secretion will be explored.

Publications

Eskin, A., Takahashi, J.S., Zatz, M., and Block, G.D.: Cyclic GMP mimics the effects of light on a circadian pacemaker in the eye of Aplysia. J. Neurosci. 4: 2466-2471, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00429-06 LCB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemistry of Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Zatz	Section Chief	SBP, LCB, NIMH
Other:	P.J. O'Brien	Section Chief	SCB, LVR, NEI
	T. Reisine	Sr. Staff Fellow	LCB, NIMH

COOPERATING UNITS *(if any)*

LVR/NEI

LAB/BRANCH

Laboratory of Cell Biology

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

a) Lithium stimulates ACTH secretion by anterior pituitary cells in culture. Lithium, phorbol esters, high extracellular calcium, and diacylglycerol comprise a distinct class of agents affecting ACTH secretion through mechanisms involving phospholipases, inositide metabolism, and protein kinase C.

b) The enzyme which transfers long chain fatty acids from acyl coenzyme A to rhodopsin has been solubilized and partially purified. This reaction is prototypical of a new class of posttranslational modification of membrane proteins, including receptors.

Project Description

Objectives: a) To determine the mechanisms by which lithium stimulates ACTH secretion from cultured anterior pituitary cells. b) To elucidate the nature and function of protein acylation.

Methods: Biochemical, chromatographic, pharmacologic, cell culture, and radioactive trace techniques.

Major Findings: a) We previously showed that lithium unexpectedly stimulated ACTH secretion and concomitantly increased inositol monophosphate (IP_1) levels in cultured anterior pituitary cells. There was no interaction between lithium and agents thought to act through cyclic AMP. Lithium pretreatment did, however, desensitize the cells to the effects of phorbol esters (PE). Active PE's are thought to specifically stimulate protein kinase C, mimicking the effects of diacylglycerols (DAG's). DAG's, together with inositol phosphates, (IP's) are the products of inositide lipid breakdown by phospholipases (which are actived by calcium). DAG pretreatment reduced lithium's effects on ACTH release and IP_1 levels. Elevated extracellular calcium stimulated ACTH release and, in the presence of lithium, markedly raised IP_1 levels. Pretreatment with DAG also attenuated these effects of calcium. Pretreatment with calcium, in turn, blocked the effects of lithium and PE on ACTH secretion. b) A number of membrane proteins, including receptors, have long chain fatty acids (usually palmitate) bound in ester linkage. We showed previously that bovine retinas incorporate [^{3}H]-palmitate into rhodopsin, the receptor for light. We are extending these observations in purified preparations. Evidence suggests that the enzyme involved is an acyltransferase which uses palmityl coenzyme A as a donor and that, like its substrate, it is an intrinsic membrane glycoprotein. After solubilization and column chromatography, specific activity is increased about 50-fold.

Significance to Biomedical Research: a) A stimulatory effect of lithium, at therapeautic concentrations, is unusual. Protein kinase C is thought to be involved in the actions of hormones and growth factors. Elucidation of the mechanism of action of lithium (and the role of protein kinase C) may shed light on the therapeautic actions of lithium as well as on the regulation of ACTH secretion (ACTH is itself an important hormone). b) Acylation of membrane proteins provides a mechanism for posttranslational modification of receptors, ion channels, etc. which could alter their function and regulate their interactions with drugs, hormones, or neurotransmitters.

Proposed Course of Project: a) Lithium's effects on protein kinase C and phospholipases will be investigated. b) Protein acyltransferase will be separated from its

substrate. Factors regulating its activity will be tested. Consequences of its activity on the physiology of rhodopsin will be sought. Acylation of other receptors and regulatory proteins will be explored.

Publications:

Butler, J. and Zatz, M.: Pantethine and cystamine deplete cystine from cystinotic fibroblasts via efflux of cysteamine-cysteine mixed disulfide. J. Clin. Invest. 77: 411-416, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00427-08 LCB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

On the Mechanism of Signal Transduction Through Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fusao Hirata, Visiting Scientist, Laboratory of Cell Biology, NIMH

See Attached Sheet

COOPERATING UNITS *(if any)*

See Attached Sheet

LAB/BRANCH

Laboratory of Cell Biology

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INSTITUTE AND LOCATION

NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glucocorticoids exert a variety of actions on various tissues and organs. These hormones act in a concert with other hormones in a permissive way. In addition, these hormones are widely used as therapeutics for inflammatory and immunological diseases; this is based upon their anti-inflammatory and immunosuppressive actions. We recently isolated lipocortin, a phospholipase inhibitory protein, from the media conditioned with glucocorticoid-treated U937 cells (a human monocyte cell line) and glucocorticoid-treated human peripheral leukocytes. Highly purified preparations of lipocortin can inhibit phospholipase A_2 in vitro and inhibit chemotaxis of leukocytes in situ, suggesting that anti-inflammatory activity of glucocorticoids is mediated through this protein. Since these preparations also alter the glycolysis in rat hepatocytes mediated through α-and β-adrenergic receptors, lipocortin is also suggested to be involved in the signal transduction of these receptors. These observations implicate that lipomodulin might mimic some of the permissive effects of glucocorticoids.

PHS 6040 (Rev. 1/84) GPO 904-917

Names, Laboratory and Institute Affiliations, and Titles of Co-principal investigator and All Other Professional Personnel Engaged on the Project.

Others: G. Kunos, Professor, McGill University
T. Ishizaka, Professor, Johns Hopkins University
K. Ishizaka, Professor, Johns Hopkins University
D. Newcomb, Professor, Johns Hopkins University
E. Gurpide, Professor, Mount Sinai Hospital
E. Schiffman, Biologist, Georgetown University
M. Nierenberg, Chief, LBG, NHLBI
S. Katz, Chief, DB, NCI
T. Shimada, Visiting Fellow, DB, NCI
G. Chrousos, Chief, DE, NICHD
J. Shelpamer, Chief, CCM, CC
Y. Wano, Visiting Fellow, LCB, NIMH
Y. Ishihara, Visiting Fellow, LCB, NIMH
R. Yamada, Guest Researcher, LCB, NIMH
C. Jelsema, Guest Researcher, LCB, NIMH
S. Kawai, Guest Researcher, LCB, NIMH
H. Tsuda, Visiting Fellow, LCB, NIMH
R. Flower, Professor, University of Bath, England
F. Russo-Marie, Associate Professor, Pasteur Institute, France

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Project Description:

Objectives: To study the biochemical mechanisms of signal transduction through receptors, including regulation of ion fluxes, cyclic nucleotides, glucose metabolism, intracellular pH, transmethylation of various components and gene expression.

Methods Employed: Enzymatic assays, spectrophotemetric assays, isotope assays, purification of proteins, radiotracer experiments, immunological techniques including immunoprecipitation, immunoblotting and radioimmunoassay, cell culture, and isolation of mRNA and DNA.

Major Findings:

In collaboration with Dr. Flower of England and Dr. Russo-Marie of France, we have recently identified several species of lipocortin; the molecular weights are 40,000, 30,000 and 15,000, respectively. The smaller species were supposed to be fragments of the 40,000 peptide. We attempted to purify these peptides from human sources such as glucocorticoids-treated U937 cells and peripheral blood leukocytes. Highly purified preparations of the 40,000 peptide were active with respect to inhibition of phospholipase A_2 *in vitro* and of leukocyte chemotaxis *in situ*. After preliminary sequencing of N-terminal amino acids, we found that human high density lipoprotein (HL A-II) is a major component of this preparation. We are trying to purify lipocortin further, using phospholipase A_2- and N-acetylglucosamine-affinity chromatography.

Highly purified preparations of human lipocortin can induce β-adrenergic receptors in lung type II cells and the adrenergic phenotype in NH15 CA2 neuroblastoma-glioma hybrid cells as glucocorticoids do. HDL-AII, a major contaminant in the preparations, has no effects and several monoclonal antibodies

raised against lipocortin can block the effects of glucocorticoids. These observations suggest that the induction of the synthesis of some proteins by glucocorticoids is a secondary effect mediated through the synthesis of lipocortin. Furthermore, the glycolysis in isolated hepatocytes is time-dependently converted from an α-adrenergic receptor mediated function to a β-adrenergic receptor mediated function. This conversion is blocked by glucocorticoids as well as by lipocortin and accelerated by anti-lipocortin antibodies. These findings suggest that lipocortin is somehow involved in the permissive effect of glucocorticoids at physiological concentrations.

Significance to Biomedical Research: Glucocorticoids are hormones from the adrenal cortex and the secretion of these hormones is known to be stimulated by physical and physiological stresses. Furthermore, patients with depression have higher serum levels of glucocorticoids and increased turnover of catecholamines. To investigate the underlying pathogenesis of these diseases, identification of mediators of glucocorticoids are essential. Lipocortin appears to be one such mediator. Furthermore, other laboratories (Prof. Ishizaka and Prof. Koltai) demonstrated that lipocortin is playing an important role in immunoregulation of IgE synthesis (allergy) and coronary and cerebral infarction (anti-ischemic action).

Proposed Course of Project: Further attempts to purify lipocortin of several molecular weights will be carried out and their properties will be compared with respect to amino acid sequence and biological activity. In addition, whether the single protein can mimic the actions of glucocorticoids or multiple factors are necessary, will be investigated.

Characterization of some cDNA clones isolated will be carried out by (a) transfection experiments, (b) sequencing of cDNA and (c) hybridization of mRNA from glucocorticoid-treated and non-treated cells. In addition, we will make efforts to isolate the normal lipocortin-cDNA from peripheral lymphocytes rather than from cancer cell lines, because regulation of synthesis and structure of protein might differ in the normal and cancer cells.

Publications:

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Hirata, F., Matsuda, K., Notsu, Y., Hattori, T., and Del Carmine, R.: Phosphorylation at tyrosine residue of lipomodulin in mitogen-stimulated marine thymocytes. Proc. Natl. Acad. Sci. (USA) 81: 4717-4721, 1984.

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Hirata, F.: Molecular mechanisms on the modulation of phospholipid metabolism by glucocorticoids. In Bailey, D. (Ed.): Prostaglandins, Leukotrienes and Lipoxins: Biochemistry, Mechanism of Action and Clinical Application. New York, Plenum Press, 1985, pp. 119-123.

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Kunos, G., Kunos, I., Hirata, F., and Ishac, E.J.: Adrenergic receptors: Possible mechanisms of inverse regulation of α- and β-receptors. J. Allergy Clin. Immunol., in press, 1985.

Notsu, Y., Namiuchi, S., Hattori, T., Matsuda, K., and Hirata, F.: Inhibition of phospholipases by Met-Leu-Phe-Ile-Leu-Ile-Lys-Arg-Ser-Arg-His-Phe, C terminus of middle-sized tumor antigen. Arch. Biochem. Biophys. 236: 195-204, 1985.

Hirata, F., Notsu, Y., Yamada, R., Ishihara, Y., Wano, Y., Kunos, I., and Kunos, G.: Isolation and characterization of lipocortin (lipomodulin). Agents and Actions, in press, 1985.

Hirata, F.: Drugs that inhibit the activities or activation of phospholipases and other acylhydrolases. In Willis, A.L., Vickerly, B.H., and Asceak, C.P. (Eds.): Handbook of Prostaglandins. Los Angeles, CRC Press, 1985, in press.

Hirata, F.: Phospholipid methylation and signal transduction. In Delgado-Escueta, A.V. (Ed.): International Symposium on Basic Mechanisms of Epilepsy. New York, Raven Press, 1985, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00434-04 LCB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Mechanisms of Action Secretion from Mouse Pituitary Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Julius Axelrod, Guest Researcher, Unit on Pharmacology, LCB, NIMH

See Attached Sheet

COOPERATING UNITS *(if any)*

See Attached Sheet

LAB/BRANCH

Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cellular mechanisms involved in adrenocorticotropin (ACTH) release were studied in a tumor cell line of the mouse anterior pituitary (AtT-20/D16-16). CRF, the natural stimulant of ACTH release, stimulates secretion through a cAMP-dependent mechanism. This was demonstrated by incorporating cAMP-dependent protein kinase inhibitor into AtT-20 cells using a liposome technique. This manipulation abolished CRF stimulated ACTH release. In contrast, K^+ and phorbol ester evoked hormone secretion was not affected by this treatment indicating that there are multiple intracellular pathways involved in regulating the release of ACTH. CRF and 8-bromo-cyclic AMP also increase the levels of proopiomelanocortin (POMC) m-RNA in AtT-20 cells. This effect is blocked by the protein kinase inhibitor indicating that CRF stimulates both ACTH release and synthesis through an activation of cAMP-dependent protein kinase.

Somatostatin (SRIF) is a potent inhibitor of ACTH release from AtT-20 cells. This peptide can block adenylate cyclase activity so as to prevent CRF and forskolin from evoking hormone secretion. SRIF also lowers intracellular calcium levels. This effect is blocked by pertussis toxin suggesting that either it is mediated by a guanine nucleotide inhibitory protein (N_i) or pertussis toxin desensitizes the SRIF receptor. Receptor binding studies show, in fact, that pertussis toxin does desensitize the SRIF receptor. SRIF also inhibits 8-bromo-cAMP and K^+ evoked ACTH release but does not affect the ability of these secretagogues to increase calcium mobilization. These data indicate that SRIF acts through multiple mechanisms to regulate ACTH secretion.

Names, Laboratory and Institute Affiliations, and Titles of Co-principal Investigator and All Other Professional Personnel Engaged on the Project.

Others: Terry D. Reisine, Senior Staff Fellow, LCB NIMH
Alberto Luini, Visiting Associate, LCB NIMH
John Kebabian, Chief, Biochemical, Neuropharmacology Section, ET, NINCDS
Genevieve Rougon, Visiting Associate, LCB, NIMH
Jacques, Barbet, Visiting Associate, LMB, NCI
Hans-Urs Affolter, Visiting Associate, LCB, NIMH
Simon Guild, Visiting Associate, ET, NINCDS

Project Description:

Objectives: To investigate the molecular events involved in ACTH release and synthesis from the anterior pituitary tumor cell line, AtT-20/D16-16. Regulation of the hormone receptors linked to ACTH release will also be examined.

Methods Employed:

Cell culture: Standard procedures for culturing both tumor cells and primary monolayers.

Biochemical: Radioimmune assays for ACTH and cyclic AMP; gel electrophoresis, protein phosphorylation techniques, ADP-ribosylation of proteins employing [^{32}P]-NAD and pertussis toxin and cellular subfractionation. Molecular biological techniques for the measurement of POMC mRNA (use of ^{32}P-labeled probe). Techniques for making liposomes. Use of antibodies against N-CAM.

Pharmacological: Ligand binding assays, superfusion apparatus to measure continuous ACTH and cyclic AMP release, Ca^{++} flux assays, Quin-2 to examine Ca^{++} mobilization.

Major Findings:

The AtT-20/D-16-16 mouse pituitary cell line is a useful system to study the molecular events involved in ACTH release since the cells are a homogenous population of corticotrophs containing a variety of hormone receptors linked to the ACTH release process. Different hormones act through a variety of second messenger systems to affect this release process. Cyclic AMP may be a second messenger in the CRF-evoked release of ACTH. CRF activates adenylate cyclase, increases cyclic AMP production, stimulates cyclic AMP-dependent protein kinase and releases ACTH. The activation of the protein kinase leads to phosphorylation of distinct cellular proteins. Incorporation of an inhibitor of the kinase blocks CRF stimulated ACTH release, indicating a direct role of this enzyme in the release ACTH. The inhibitor protein does not block K^+ or phorbol ester evoked hormone secretion implying that there are multiple pathways involved in releasing ACTH.

In addition to stimulating the secretion of ACTH, CRF increases the synthesis of this polypeptide. CRF increases the levels of proopiomelanocortin (POMC) m-RNA in AtT-20 cells. This is most likely due to an activation of the POMC gene. 8-bromo-cAMP also increases POMC mRNA levels and the effect of both CRF and 8-bromo-cAMP is prevent by the inhibitor of cAMP dependent protein kinase. Therefore, this enzyme is involved in both the regulation of ACTH release and synthesis by CRF.

While several hormones evoke ACTH release, the hypothalamic peptide somatostatin (SRIF) is a potent inhibitor of ACTH secretion. SRIF acts through multiple mechanisms to prevent ACTH release. Studies employing pertussis toxin have shown that SRIF acts through a guanine nucleotide inhibitory protein (N_i) to reduce the ability of secretagogues to increase cyclic AMP accumulation. SRIF also lowers basal calcium levels but it does not prevent 8-bromo-cAMP from increasing cytosolic calcium concentrations. Pertussis toxin reduces SRIF's inhibition of 8-bromo-cAMP and K^+ evoked ACTH secretion. This is due to the toxin's ability to desensitize SRIF receptors. This was shown in ligand binding studies. Thus, N_i not only couples SRIF receptor to adenylate cyclase but also regulates the sensitivity of SRIF receptors so as to affect non-adenylate cyclase second messenger systems.

Significance to Biomedical Research: The release of ACTH is an important response of the body to stress. By studying the mechanisms by which different hormones regulate ACTH secretion and synthesis, it may be possible to understand at a cellular level the events involved in stress and the possible molecular abnormalities associated with chronic stress.

Proposed Course of Project: Future studies will be directed to elucidating the various intracellular events involved in ACTH release and synthesis. Identification of the phosphoproteins regulated by CRF and their role in ACTH synthesis and secretion will be attempted. The precise mode of regulation of POMC gene activity by CRF will also be attempted.

Publications:

Miyazki, K., Reisine, T. and Kebabian, J.: Cyclic AMP-dependent protein kinase activity in rodent pituitary tissue: possible role in cAMP dependent hormone secretion. Endocrinol. 115: 1933-1945, 1984.

Reisine, T., Zhang, Y. and Sekura, R.: Pertussis toxin treatment blocks somatostatin's inhibition and increases forskolin's stimulation of cyclic AMP accumulation and adrenocorticotropin secretion from mouse anterior pituitary tumor cells. J. Pharmacol. Expt. Therap. 232: 275-282, 1984.

Reisine, T.: Somatostatin inhibition of cyclic AMP accumulation and adrenocorticotropin release: Mechanism of action and mode of self regulation. CINP Congress, symposium on "Receptors and specific binding sites in the brain", Florence, Italy, 1985, in press.

Reisine, T.: Somatostatin inhibition of cyclic AMP accumulation and adrenocorticotropin release from mouse anterior pituitary tumor cells; Mode of action and self-regulation. In Cooper, D. and Seamon, K. (Eds.): Advances in Cyclic Nucleotide and Protein Phosphorylation, New York, Raven Press, 1984, pp. 57-65.

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Luini, A., Goldberg, O., and Teichberg, V.: Differential sensitivity of selected brain areas to excitatory neurotransmitters released by potassium depolarization. Neurosci. Letters, 49: 325-330, 1984.

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Luini, A., Corda, D., and Axelrod, A.: Press. Secretagogues elevate cytosolic calcium by stimulating cAMP formation in a corticotropin secreting cell line. Regulatory Peptides, in press, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00881-29 LCM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intermediary Energy Metabolism in Mammalian Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Elaine E. Kaufman	Research Chemist	LCM, NIMH
Others:	Thomas Nelson	Senior Staff Fellow	LCM, NIMH
	Louis Sokoloff	Chief	LCM, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	2.25	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project described in this report has involved two general areas: 1) the identification of and regulatory mechanisms involved in the biosynthetic and degradative pathways for γ-hydroxybutyrate (GHB), a naturally occuring compound in mammalian brain which is thought to function either as a neuromodulator or as a neurotransmitter, and 2) the study of certain pharmalogical effects of GHB especially those effects which bear a close resemblance to those of opiates such as morphine.

The focus of this project will now be on the role of GHB at the cellular level in the central nervous system with an emphasis on the biochemical events which mediate its action.

Project Description:

The discovery that administration of GHB could alter the levels of serotonin, acetylcholine and dopamine in the CNS as well as the function of dopaminergic neurons, led to the proposal of a neuromodulatory role for GHB. More recently, the discovery of high affinity binding sites for GHB as well as specific uptake and release systems have led to the additional proposal that endogenous GHB may function as a neurotransmitter in the CNS.

In as much as both synthesis and degradation play an important role in regulating the concentration of GHB at its site of action, the study of both of these processes has been until now an important part of this project. We have purified and characterized an enzyme (GHB dehydrogenase) capable of catalyzing the first step in the degradative pathway. This enzyme has been shown to have properties which would allow it to function under physiological conditions.

We have found a number of endogenous compounds which inhibit GHB dehydrogenase *in vitro*. Some of these inhibitors when administered *in vivo*, resulted in elevated tissue levels of GHB presumably by inhibiting GHB dehydrogenase activity in the cells. We have also found a second group of aldehydic substrates for GHB dehydrogenase of which D-glucuronate is a representative which can increase the rate of GHB oxidation *in vitro* by allowing the pyridine nucleotide cofactor ($NADP^+$) to function catalytically and thus avoiding inhibition by NADPH. Infusion of D-glucuronate *in vivo* results in a substantial decrease in the half-life of exogenous GHB in plasma.

Not only certain biological intermediates but also common drugs such salicylate and anticonvulsant drugs such as sodium valproate are excellent inhibitors of GHB dehydrogenase and when administered to experimental animals lead to elevated tissue levels of GHB. The elevation of GHB in brain following administration of valproate has preivously been attributed to inhibition of succinic semialdehyde dehydrogenase; however, a comparison of the Ki's for valproate and salicylate for GHB dehydrogenase and succinic semialdehyde dehydrogenase indicates that the affinity of GHB dehydrogenase for these drugs is 10 to 100 times greater than that of SSA dehydrogenase. Therefore, it is likely that it is the inhibition of GHB dehydrogenase which is leading to the elevated tissue levels of GHB.

The preparation of an antibody to GHB dehydrogenase has allowed us to 1) identify the cytosolic enzyme which degrades GHB to succinic semialdehyde as GHB dehydrogenase, and 2) to determine that a mitochondrial enzyme which also catalyzes the conversion GHB to succinic semialdehyde is not GHB dehydrogenase.

Pathways for the biosynthesis of GHB appear to differ between brain on the one hand and kidney and muscle on the other. GABA appears to be the precursor in all of these tissues; however, in brain the GABA is derived from glutamate whereas in kidney and muscle, putrescine is the precursor. *In vivo* studies in which inhibitors of the polyamine pathway such as difluoromethylornithine or pargyline have been administered have led to lower

concentrations of GHB in kidney but not in brain where GABA is derived from glutamate.

Since a number of similarities between the physiological effects of both morphine and the naturally occurring opiates and GHB have been reported, we have examined the effect of naloxone on the reduction in cerebral glucose utilization. This study showed that naloxone could significantly reverse this decrease in glucose utilization in some of the structures which were examined. We have also found that the dose response curve for the effect of GHB on body temperature is remarkably similar to that of morphine, with hyperthermia resulting from low doses of GHB and hypothermia from high doses of GHB. The doses at which maximum hyperthermia occur are lower than those at which other pharmacological responses have been observed and are within the range of endogenous tissue levels of GHB.

Significance to Biomedical Research and to the Program of the Institute:

Our work has focused on the elucidation of the pathways of biosynthesis and degradation of GHB, a putative neurotransmitter in the CNS, and on the factors which regulate these pathways. We are now beginning a study of the biochemical and physiological responses which mediate the pharmacological actions of GHB. These studies may aid (1) in understanding both the metabolic and physiological disturbances which are seen in patients with γ-hydroxybutyric aciduria, (2) in understanding the action as well as the disposition of the GHB now being administered to some patients with narcolepsy, and (3) in evaluating the high levels of GHB found in certain areas of the brain of patients with degenerative diseases such as Huntington's Chorea as well as the very high levels found in normal fetal brain.

Proposed Course:

This project will now focus primarily on studies directed toward an understanding of the role of GHB in the central nervous system with particular attention to the function of GHB at the cellular level.

1. We will continue our investigation of the relationship of GHB to the opiates and investigate the question of whether GHB has opiate-like properties or is activating an endogenous opiate system.

2. Pharmacological studies designed to investigate the effects of low doses of GHB will continue.

3. An investigation of the biochemical basis for the pharmacological actions of low doses of GHB will be started. The effect of low doses of GHB on prostaglandin synthesis, suggested by the hyperthermic effects of low doses of GHB, will be studied as will the effect of GHB on calcium uptake and release, a study also suggested by the similarities of GHB to the opiates.

Publications:

Nelson, T., Kaufman, E.E., and Sokoloff, L.: 2-Deoxyglucose incorporation into rat brain glycogen during measurement of local cerebral glucose utilization by the 2-deoxyglucose method. J. Neurochem. 43: 949-956, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00882-18 LCM

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Regional Cerebral Circulation and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Louis Sokoloff	Chief, Lab. Cerebral Metabolism	LCM, NIMH
Others:	Charles Kennedy	Guest Researcher	LCM, NIMH
	Thomas Nelson	Senior Staff Fellow	LCM, NIMH
	Linda Porrino	Senior Staff Fellow	LCM, NIMH
	Carolyn B. Smith	Senior Staff Fellow	LCM, NIMH
	Gerald A. Dienel	Staff Fellow	LCM, NIMH

COOPERATING UNITS *(if any)*

Theoretical Statistics & Mathematics Branch, NIMH; Biological Psychiatry Branch, NIMH; Lab. Cell Biology, NIMH; Lab. Neurophysiology, NIMH; NINCDS, NIH

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12.0	5.5	6.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A method has been developed for the quantitative determination of the rates of local glucose consumption in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of [^{14}C]deoxyglucose as a tracer for glucose flux through the hexokinase step. Local [^{14}C]deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping metabolically, and therefore functionally, linked structures in the CNS, the method is being used to study alterations in the energy metabolism of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states.

OTHER INVESTIGATORS (CONTINUED)

Giovanni Lucignani	Visiting Fellow	LCM, NIMH
Therese M. Jay	Visiting Fellow	LCM, NIMH
Kentaro Mori	Visiting Fellow	LCM, NIMH
Floyd R. Domer	IPA	LCM, NIMH
Irwin Kopin	Director	IRP, NINCDS
Richard Burns	Professor, Vanderbilt Medical School, Nashville, Tennessee	
Astrid Nehlig	Chargee de Recherche CNRS, Nancy, France	

Project Description:

Previous work in this Laboratory led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{14}C]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different inasmuch as the transport carrier and the enzyme discriminate kinetically between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

All the theoretical and technical problems were solved, and the method has now been completely operative for the last eight years. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of [^{14}C]deoxyglucose is injected, and arterial plasma concentrations of [^{14}C]deoxyglucose and glucose are measured from the time of injection until 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue [^{14}C]deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer. The method has now been in use for at least seven years in this Laboratory and in laboratories around the world. It has been found to be generally successful but requires adaptation to certain types of pathophysiological states, such as severe hypoglycemia, status epilepticus, and ischemia, when the balance between glucose supply and glucose consumption in brain is disturbed. In these cases, the method is still valid, but the rate constants and lumped constant (components of the operational equation) have to be recalibrated for the specific condition.

Numerous applications of the method to physiological and pharmacological conditions have been made in this Laboratory and published in recent years. A couple more of such studies have been continued and completed the last year.

Recently, an animal model of Parkinson's disease has been developed by administration of N-methyl-4-phenyl-1236 tetra-hydro-pyridene (MPTP) to primates. This drug specifically destroys the cells of the substantia nigra pars compacta, and the monkeys have all the major clinical features of Parkinson's disease in humans. Dr. Linda Porrino in collaboration with Dr. Richard Burns and Dr. Irwin Kopin (NINCDS) have applied the deoxyglucose method to monkeys treated with MPTP and to MPTP-treated monkeys receiving L-DOPA therapy. Glucose utilization in monkeys made Parkinsonian when compared to glucose utilization in normal monkeys was found to be altered in some areas of the basal ganglia, most prominently in the substantia nigra pars compacta where dopamine cells had been destroyed as well as in the subthalamic nucleus and the external segment of the globus pallidus. No changes were found in dopaminergically innervated limbic areas such as the nucleus accumbens or lateral septum, or in thalamic and cortical regions. Glucose utilization in monkeys receiving L-DOPA therapy following MPTP treatment was increased throughout areas of motor and premotor cortex as well as in motor nuclei of the thalamus, ventral anterior and ventrolateral nuclei. The most prominent increases were found in the subthalamic nucleus and in the globus pallidus.

These changes in glucose utilization are in sharp contrast to the lack of effects on energy metabolism seen following the administration of L-DOPA to normal animals. These results are significant for two reasons. First, they place emphasis on the role of the globus pallidus-subthalamic nucleus circuit in the organization and performance of normal movement. Second, these results provide insights into the therapeutic actions of L-DOPA in Parkinson's disease, by clearly demonstrating the differences in this drug's actions in normal and diseased brain. Further work on this collaborative project will concentrate on the role of the subthalamic nucleus in movement disorders and on the modes of action of other therapeutic agents used in the treatment of Parkinson's disease.

In another study Dr. Astrid Nehlig and Dr. Linda Porrino studied brain energy metabolism during the estrus cycle in female rats. Glucose utilization in the brain of female rats displayed cyclic variation with the highest levels evident during proestrus and metestrus stages of the cycle. Significant changes were found in the hypothalamus, particularly in the preoptic and arcuate areas and in some limbic structures. Comparison of the values obtained in females to those seen in males revealed significant differences in rates of glucose utilization in areas known to be involved in control of sexual behavior. Rates in most structures, however, did not differ in males and females. These studies were completed during the past year, and a manuscript describing them is currently in press.

The deoxyglucose method measures the amount of 2-deoxyglucose-6-phosphate which is formed over a period of 45 minutes following an injection of 2-deoxyglucose. The rate at which this analogue accumulates is related to the rate of glucose metabolism during that interval. The method has been challenged in some circles since its publication in 1977 on the grounds that it fails to account for loss of label as a result of the action of glucose-6-phosphatase, an enzyme generally considered to be absent or negligible in brain. Underestimation of the activity of this enzyme would result in values for glucose utilization which would be too low. Despite the close agreement between values for glucose metabolism of the whole brain obtained by the standard Kety-Schmidt method and the deoxyglucose method, the controversy over the effect of glucose-6-phosphatase in the brain continues. In view of the potential impact that these recurrent criticisms may have on the use of the method, it has been necessary to undertake a detailed experimental investigation of the validity of these criticisms.

The laboratory has completed a major and time-consuming project designed to deal with the most frequent and persistent criticisms of the deoxyglucose method. The issue is whether the brain has sufficient glucose-6-phosphatase activity to dephosphorylate deoxyglucose-6-phosphate during the period of time required by the deoxyglucose method for measuring cerebral glucose utilization. If glucose-6-phosphatase activity in brain is sufficiently high, then the deoxyglucose method (which assumes no active dephosphorylation of deoxyglucose for 45 minutes after a pulse of deoxyglucose) will underestimate the true rate of cerebral glucose utilization. Despite the close agreement between values for glucose metabolism obtained by the standard Kety-Schmidt method and the deoxyglucose method, the controversy over the effect of glucose-6-phosphatase in brain continues.

Two papers critical of the deoxyglucose method have been published in the last 3 years. The first paper by Huang and Veech, J. Biol. Chem. 257: 11358-11363, 1982, purported to demonstrate that brain dephosphorylated glucose-6-phosphate at a rate equal to 35% of the rate of glucose phosphorylation. Briefly, Huang and Veech reported that when [U-^{14}C]glucose mixed with [2-^{3}H]glucose was injected into rats through the carotid artery, there was a progressive and rapid loss of ^{3}H but not of ^{14}C in the glucose pool in brain. No significant differential rate of loss of label was observed in blood during this interval. Inasmuch as G-6-P is in rapid equilibration with F-6-P which loses the 2-^{3}H label, but not its ^{14}C label, any decline in the ^{3}H/^{14}C ratio from that in the injectant would indicate that glucose was being reformed by the hydrolysis of G-6-P.

Experiments performed in this laboratory by Dr. Thomas Nelson and most others have conclusively shown that the report from Huang and Veech was wrong. The pure glucose fraction extracted from brain after a pulse of a mixture of [2-^{3}H]glucose and [U-^{14}C]glucose does not have a differential rate of loss of ^{3}H over U-^{14}C in brain greater than that of the pure glucose fraction extracted from the blood which perfuses the brain.

Further experiments conducted in this laboratory by Dr. Gerald Dienel have shown that the glucose fraction extracted from tissue as described by Huang and Veech is contaminated with other compounds which are the source of the low ^{3}H/^{14}C ratios. The claims of Huang and Veech that the activity of glucose-6-phosphatase in brain is very active are without foundation. A paper refuting the claims by Huang and Veech has recently been published in Science. Experiments designed to identify the major source of contaminating compounds in the brain glucose fraction of Huang and Veech are being concluded by Dr. Dienel. No further work on this problem is anticipated.

The second paper criticizing the deoxyglucose method came from Sacks et al., Neurochem. Res. 8: 661-685, 1983. The author of this paper has been active in trying to discourage the use of the deoxyglucose method and has raised doubts in the scientific community concerning the validity of the deoxyglucose method. Each of the criticisms by Sacks et al. of the deoxyglucose method has been carefully examined and has been shown to be without foundation.

All of Sacks' major criticisms of the deoxyglucose method come from experimental observations which he made after infusing labeled deoxyglucose into rats. Three different experiments are described which supposedly show that: 1) there is no increase in 2-deoxyglucose-6-P concentration in brain with time after an i.v. injection of deoxyglucose. (The continued increase in deoxyglucose-6-phosphate in brain is a mandatory requirement of the deoxyglucose method.) 2) Four minutes after a pulse of a mixture of 2-[1-^{14}C]deoxyglucose and [1-^{3}H]glucose, there is a net loss of 2-deoxyglucose from the brain while there is no net loss of glucose into the venous blood. 3) [^{14}C]glucose-6-phsophate is taken up by the brain, where it is dephosphorylated and returned to the cerebral venous blood as free glucose.

Experiments in this laboratory by Drs. T. Nelson and G. Lucignani have shown Sacks' first criticism to be wrong. Deoxyglucose-6-phosphate accumulates

in the brain for 45 minutes following an intravenous pulse of deoxyglucose. This observation required the use of a brain blower which removes and freezes brains within less than 1 second. Sacks removed brains by dissection which is too slow to prevent postmortem phosphorylation from occurring.

Sacks' second observation is correct but completely predictable from the model of the deoxyglucose method. Simulations performed in this laboratory show that the differences in postinfusion arteriovenous differences for glucose and deoxyglucose are entirely explained by the known differences in the rate constants for phosphorylation of glucose and deoxyglucose.

The third observation of Sacks et al. is experimentally invalid. Three types of experiments in this laboratory show that glucose-6-phosphate does not cross the blood brain barrier in the mature rat. If rats are infused with [^{32}P]glucose-6-phosphate so as to achieve an approximately constant concentration of [^{32}P]glucose-6-phsophate, there is no appreciable arteriovenous difference for glucose-6-phosphate across the brain. Brains from these experiments were assayed for ^{32}P and all of the ^{32}P found in the brain was fully accounted for by the radioactivity in the blood trapped in the brain. There was no evidence of uptake of [^{32}P]glucose-6-phosphate by the brain. The Crone single pass capillary extraction technique was used as the last method of showing that glucose-6-phosphate does not cross the blood barrier. By this technique it was shown that the ratio of the concentrations of glucose-6-phosphate and sucrose in the mixed cerebral blood draining from the brain was unchanged from the ratio of the two compounds in the arterial blood. Glucose-6-phosphate is not taken up by brain any better than is the reference compound--sucrose which does not cross the blood brain barrier.

A manuscript which rebuts the criticisms of Sacks et al. has been submitted to the Journal of Neurochemistry. There is no further work planned on this project.

Significance to Biomedical Research and to the Program of the Institute:

The deoxyglucose method has made it possible for the first time to measure the rates of glucose utilization simultaneously in all functional and structural components of the central nervous system of conscious, behaving animals and now also in man. Because the method was developed in our Laboratory, it has been our responsibility to survey its applicability to the various types of conditions in which it might be applicable. The program has, therefore, been somewhat heterogeneous covering a wide range of physiological, pharmacological, pathological, and altered behavioral states. The method and its wide-ranging usefulness has now been more or less established, and it is used extensively throughout the world in neuroanatomical, neurophysiological, neuropharmacological, psychiatric, neurological, and neurosurgical research, and its wide acceptance is directly related to the results of studies in this project.

Proposed Course:

Applications of the deoxyglucose method to problems of neurophysiology, neuropharmacology, neurology, and psychiatry will be continued. A project is in the planning stage to adapt the method for use in neuropathological conditions such as stroke, status epilepticus, etc. Efforts will be made to improve the quantitative resolution of the method to the single cell and subcellular levels. Immunocytochemical techniques will be introduced with the aid of Dr. Bernard Driscoll to correlate local cerebral rates of glucose utilization with local levels of neuropeptides and the host of putative neurotransmitters and neuromodulators. A cell culture facility will be established in the Laboratory by Dr. Driscoll to allow studies of cellular mechanisms of carbohydrate transport across cell membranes which are necessary to examine the rates of glucose utilization in neuronal and glial cellular components of the cerebral tissue.

Publications:

Sokoloff, L.: Editorial - Message to the membership from the retiring president of the International Society of Cerebral Blood Flow and Metabolism. J. Cerebral Blood Flow Metab. 3: 405-406, 1983.

Sokoloff, L.: Metabolic probes of central nervous system activity in experimental animals and man. Magnes Lecture Series, Vol. I. Sunderland, MA, Sinauer Associates, Inc., 1984, pp. 1-97.

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Hökfelt, T., Smith, C.B., Norell, G., Peters, A., Crane, A., Goldstein, M., Brownstein, M., and Sokoloff, L.: Attempts to combine 2-deoxyglucose autoradiography and tyrosine hydroxylase immunohistochemistry. Neuroscience 13: 495-512, 1984.

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Sokoloff, L.: Localization of functional activity in the central nervous system by metabolic probes. In Paton, W., Mitchell, J., and Turner, P. (Eds.): Proceedings, IUPHAR 9th International Congress of Pharmacology. London, Macmillan Press Ltd., 1984, Vol. 1, pp. 9-29.

Sokoloff, L. and Smith, C.B.: Basic principles for assay of local cerebral metabolic rates in vivo. In Porter, R.J. et al. (Eds.): Advances in Epileptology: XVth Epilepsy International Symposium. New York, Raven Press, 1984, pp. 69-77.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00887-08 LCM

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Extended Visual System of the Macaque Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles Kennedy	Guest Researcher	LCM, NIMH
Other:	Louis Sokoloff	Chief	LCM, NIMH
	Mortimer Mishkin	Chief	LN, NIMH
	Jocelyn Bechevalier	Visiting Associate	LN, NIMH

COOPERATING UNITS *(if any)*

Laboratory of Neuropsychology, NIMH

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.25	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The deoxyglucose method is being applied to the monkey to advance knowledge regarding the parts of the brain which are involved in the process of visual information. By measuring rates of local cerebral glucose utilization in animals during their performance of tasks involving different types of visual stimuli we anticipate learning which parts of brain are involved in such functions as discrimination, memory and motivation. Also by studying animals at various ages, information will be obtained regarding the maturation of the visual processing system.

An extension of this project is an examination of the parts of brain which participate in motor activity. This is possible because the task employed in response to visual stimulation involved repetitive lever pressing of one hand. Knowledge of loci involved in motor activity is of importance to those interpreting the results of studies with ^{18}fluorodeoxyglucose and PET scanning. Because of the far greater resolution of the ^{14}C deoxyglucose method our studies will be a standard for comparison with the clinical procedure.

Project Description:

A collaboration was initiated in 1978 which was designed to map cortical regions of brain which were responsive to visual stimulation. This was a logical sequel to the mapping of the primary visual system which had been among the first applications of the deoxyglucose method. The procedure followed in the collaboration was the preparation of animals which had one hemisphere deprived of visual input. This was accomplished by section of one optic tract, the corpus callosum and forebrain commissures. When the deoxyglucose method is applied to animals prepared in this manner during their performance of a task dependent upon visual cues, it was possible to map the limits of a wide expanse of cortical regions which are vision related, by noting side to side differences in rates of glucose utilization in homologous cortical regions. The results of these studies in mature animals have been published.

The functional development of the extended cortical visual system is being studied by preparing infant monkeys with unilateral optic tract section and forebrain commissurotomy. The deoxyglucose method is then employed in the manner described for the mature animals. From a knowledge of the maturational characteristic of visual function the age range for these experiments was chosen to be 1 day to 5 months. The results show that there are systematic, age-related changes in rates of glucose utilization in normal visual related cortex as well as right-left differences between intact and visually deprived cortex. In all cortical visual areas of the intact hemisphere glucose utilization was lowest in the youngest subjects and reaches a maximum at four months. A single study at six months of age is at adult levels suggesting that the lower, mature rate is achieved long before pubescence. As in adult monkeys, the intact hemisphere of infants shows a progressive decline in glucose utilization along the ventral cortical visual pathway. This gradient was seen in all animals but was shallowest in the two youngest. The deprived hemisphere had low rates of glucose utilization compared to the intact hemisphere at all ages. The differences were greatest in the primary visual cortex (area OC) and smallest in the most anterior temporal cortex (area TE). These differences varied systematically with the age of the animal with maturation being accompanied by a serial increase of the intact-deprived difference until four months of age when it reached a maximum. This age of the peaking of both the absolute rate in the intact cortex and the intact-deprived difference corresponds with the age when the animal attains the capacity for object recognition (Bachevalier and Mishkin, Int. J. Psychophysiol., 1983).

In the experiments cited above many animals had been trained to respond to a specific visual stimulus with unimanual key-pressing to obtain a water reward. Thus the same experiments which were used to map the extended visual system also provided information on the metabolic responses to motor activity. While the motor pathways of the brain have been identified by other techniques, and thus are known, these experiments served to delineate the specific subdivisions of many structures which selectively are activated in the unimanual key-pressing. They provided new information on somatotopic localization of arm-hand movements. This was particularly well-defined in the study of cerebellar cortex. A large part of Crus II of the ipsilateral cerebellar cortex was shown to be selectively

responsive in the animals' performance of the task. Also participating, but with a lesser percent change, was the lateral portion of the vermis in lobules III-VI. Localized increments in the rate of glucose utilization were also noted in VL and VPL of the thalamus, part of the globus pallidus and discrete zones of cerebral cortex (S, S_{II}, M) and a part of the supplementary motor area. A noteworthy feature of this mapping study of motor activity is that a much greater metabolic increment was found in structure concerned with sensory monitoring of motor activity than in those related to the motor activity itself.

The wealth of information with respect to brain structures involved in motor activity has been obtained by a rough sampling of regions whose asymmetrical concentrations of ^{14}C are apparent from inspection of autoradiographs. A much more detailed survey designed to delineate both the exact limits of participating regions is now underway with the aid of the image processing system. This, while far more time consuming than sampling involved regions by manual densitometry, promises to give a far more inclusive and detailed picture of motor related functional regions. It is planned to extend the analysis to awake, inactive controls and to sleeping animals. This comparison may yield useful information with respect to delineating regions which are not confined to one hemisphere but which are activated as a result of the mental state of concentration and attention to the conduct of a task.

Significance to Biomedical Research and to Program of the Institute:

This project represents a collaborative effort between the Laboratory of Cerebral Metabolism and the Laboratory of Neuropsychology in which the specialized expertise of each Laboratory is brought to bear on the use of the deoxyglucose method to study higher nervous functions, in this case the higher level processing of visual information and task performance involving motor activity. The advantage of this approach is the ability to examine all local regions of the brain simultaneously in unanesthetized animals. It is hoped that these studies will help to elucidate the regions of the brain involved in integrating sensory inputs and motor activity.

Proposed Course:

The analysis of data obtained in experiments already carried out will be continued. To establish age related changes accompanying maturation more monkeys will be added to the series. These will be 3-6 months of age. Yet to be undertaken are experiments with different types of visual stimulation and with visual stimulation associated with tasks requiring learning and memory.

The Laboratory of Neuropsychology is reporting on this project with Report No. Z01 MH 02033-08 LN, titled "Functional Mapping of Sensory Systems".

Publications:

Kennedy, C.: Metabolic mapping of the primary visual pathway. In Sokoloff, L. (Ed.): Brain Imaging and Brain Function: Association for Research in Nervous and Mental Disease. New York, Raven Press, 1985, Vol. 63, pp. 61-72.

Macko, K.A. and Mishkin, M.: Metabolic mapping of higher order visual areas in the monkey. In Sokoloff, L. (Ed.): Brain Imaging and Brain Function: Association for Research in Nervous and Mental Disease. New York, Raven Press, 1985, Vol. 63, pp. 73-86.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00889-06 LCM

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Method for the Determination of Local Rates of Protein Synthesis in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Carolyn B. Smith	Chemist	LCM, NIMH
Others:	Louis Sokoloff	Chief	LCM, NIMH
	Charles Kennedy	Guest Researcher	LCM, NIMH
	Kentaro Mori	Visiting Fellow	LCM, NIMH
	Nancy Eng	Chemist	LCM, NIMH
	Mortimer Mishkin	Chief	LN, NIMH
	W. Mendelson	Research Psychiatrist	CPB, NIMH
	R. K. Nakamura	Senior Staff Fellow	LPP, NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, NIMH; Lab. of Psychology and Psychopathology, NIMH; Lab. of Neuropsychology, NIMH

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. The method is based on the use of L-[1-^{14}C]leucine as a tracer for the incorporation of leucine into protein. Six kinetic models for the behavior of leucine on brain have been designed. By mathematical analysis of the kinetics of exchange of the amino acid between plasma and the tissue pool(s) and its incorporation into protein, equations have been derived for each model that define the rate of amino acid incorporation into protein in terms of the time course of plasma-specific activity, final tissue concentration of ^{14}C, and experimentally determined kinetic constants. Tissue concentrations of ^{14}C are determined by quantitative autoradiography. Experiments are being carried out to test the validity of the various models.

The method is currently being applied to studies of aging, development, hypothyroidism, regeneration, and sleep.

Project Description:

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. This method is similar to the [^{14}C]deoxyglucose method in that it is based on enzyme kinetic principles as applied to the measurement of reaction rates in vivo with labeled tracers as substrates. The two requirements for this type of method are: 1) label must be primarily in the form of labeled product (labeled protein) at the end of the experiment, and 2) a model for the behavior of the precursor in brain in order to calculate the integrated specific activity of the precursor at the site of the reaction in terms of measurable variables. In order to satisfy the first requirement [1-^{14}C]L-leucine has been chosen as the labeled tracer. The only metabolic pathway for leucine apart from protein synthesis entails a transamination followed by a decarboxylation. Therefore, in the metabolism of [1-^{14}C]leucine the label is transiently transferred to α-ketoisocaproic acid and ultimately to $^{14}CO_2$ which is then diluted in the large CO_2 pool in brain produced by carbohydrate metabolism. There are, therefore, no residual radioactive products other than labeled protein. In order to meet the second requirement we have designed kinetic models for the behavior of leucine in brain. All of the models are based on the following assumptions and requirements:

1) Homogeneous tissue compartments.
2) No isotope effect.
3) No release of labeled leucine from labeled protein during the 60 minute experimental period.
4) Complete loss of label from metabolic degradation of leucine.
5) Steady state for unlabeled leucine.

We have developed six such models, and by mathematical analysis we have derived operational equations for all of them. The models are of progressively increasing complexity as regards the number of compartments and the interrelationships among them. Regardless of the degree of complexity of the model used, the operational equation has the same general form. The numerator is composed of the total amount of radioactivity in the tissue at the end of the experiment minus term(s) for the amount of radioactivity in any of the tissue pools of free leucine. The denominator is composed of the integrated plasma specific activity minus term(s) for the lag between the precursor pool and the plasma. Optimal design of the experimental procedure allows us to reduce this expression to thetotal radioactivity in the tissue divided by the integrated plasma specific activity. The terms in the numerator for the free leucine pools can be eliminated by fixing and washing tissue sections. The expression for the lag in the denominator can be made very small by administering a pulse of [^{14}C]leucine and waiting 60 minutes estimated to be more than 10 half lives of the precursor pool in white matter.

The major question remaining is that of how much admixture there is of leucine derived from protein degradation and the precursor pool. We are currently trying to determine the fraction of leucine in the precursor pool that is derived from the plasma. The experiment consists of the determination of the specific activity of brain leucyl-tRNA and plasma leucine in a rat in a steady state for both labeled and unlabeled leucine in the plasma. If all of the

leucine is derived from the plasma, the specific activity of the leucyl-tRNA will eventually reach that of the plasma. If there is a significant contribution of leucine from protein degradation, the ratio of the specific activities of leucyl-tRNA and plasma leucine will be a measure of the fractional contribution from plasma.

The method has been worked out for a programmed infusion of labeled leucine designed to maintain a constant plasma level. The microchemical analytical and separation technique for the determination of picomolar levels of leucine have been developed and are being tested.

Meanwhile with the assumption that there is no admixture of leucine derived from protein degradation, we are using the method for the determination of, at worst, minimal local rates of cerebral protein synthesis. We have determined local rates of protein synthesis in normal, conscious male rats. We have carried out studies of the effects of focal seizures, axotomy of a cranial nerve, and chronic hypothyroidism in rats. The results show that these conditions all result in specific changes in cerebral protein synthesis and that the [^{14}C]leucine method has the sensitivity to detect such changes.

In collaboration with the Unit on Sleep Studies, and the Laboratory of Neuropsychology, we are also studying the effects of slow wave sleep on local rates of protein synthesis in monkey. This study is designed to test a long held hypothesis that sleep is a physiologic period during which the brain tissue undertakes repair and remodeling. To date 4 awake and 4 asleep monkeys have been studied. Unfortunately in the last 2 animals plasma leucine levels were unusually high, and it will be necessary to add an additional 2 animals to this series. In the 3 awake and 3 asleep monkeys local rates of protein synthesis have been determined in 80 brain regions with the use of the computer-assisted image-processing system. These preliminary results show a general increased rate of cerebral protein synthesis throughout the brain. Statistically significant increases occur in 7 of the brain regions examined including the pulvinar, lateral habenula, interpeduncular nucleus, magnocellular reticular nucleus, dorsal motor nucleus of the vagus, claustrum and accessory auditory cortex.

Significance to Biomedical Research and Program of the Institute.

Protein synthesis is probably the most important biochemical process underlying the development, maturation, plasticity, maintenance, and long-term regulation of the nature and degree of functional activity of the nervous system. The structural, functional, and metabolic properties of the tissues largely reflect the role of structural and enzymatic proteins. Peptides that are considered to be neurotransmitters are in some, and possibly all, cases derived from the cleavage of large parent protein molecules. Many hormones within and outside the nervous system are proteins. It is, therefore, certain that changes in protein synthesis can and do alter function and that some mental and neurological dysfunctions reflect disturbances in this vital biochemical process. This research is directed at determining the rates of protein synthesis in specific regions of the central nervous system with an ultimate resolution down to the cellular level. This provides for the first time the opportunity to study

at the individual structural or anatomical level the changes in protein synthesis that may be the causes, consequences, or correlates of normal conditions, such as maturation, plasticity, differentiation, sleep, learning and memory, behavioral patterns, etc., or pathological conditions, such as hormonal disorders, aging, regeneration in response to injury, convulsive disorders, coma, etc.

Proposed Course.

We are continuing our efforts on completing experiments on the question of dilution of the precursor pool specific activity with leucine derived from protein degradation. In the same experiments we are determining again the half-life of the precursor pool. The applications of the method to the study of slow wave sleep will be continued.

Publications:

Smith, C.B.: The influence of age on cerebral energy metabolism. In Energy Transduction and Neurotransmission, Joint ESN-WFN Symposium, Rome, September 20-21, 1982. (In press) 1985.

Sokoloff, L., and Smith, C.B.: Basic principles underlying radioisotopic methods for assay of biochemical processes in vivo. In Greitz, T., Ingvar, D.H., and Widen, L. (Eds.): The Metabolism of the Human Brain Studied with Positron Emission Tomography. Raven Press Books, Ltd., London, 1985, pp. 123-148.

Smith, C.B., Crane, A.M., Kadekaro, M., Agranoff, B., and Sokoloff, L.: Stimulation of protein synthesis and glucose utilization in the hypoglossal nucleus induced by axotomy. J. Neurosci. 4: 2489-2496, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02216-02 LCM

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic Mapping of the Brain during Rewarding Self-Stimulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda J. Porrino	Senior Staff Fellow	LCM, NIMH
Others:	Ralph U. Esposito	Senior Staff Fellow	LCM, NIMH
	Louis Sokoloff	Chief	LCM, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The deoxyglucose method is being used to study alterations in local cerebral glucose utilization in rats during the performance of goal-oriented self-stimulation behavior. By mapping metabolic activity in rats during electrical stimulation to various discrete areas of the brain, information can be obtained about those areas of the brain involved in motivation, learning, and reinforcement of behavior. Also studying the effects of drugs of abuse such as the psychostimulants and opiates with the deoxyglucose method will provide information about the areas in the brain which mediate the reinforcing properties of these drugs.

Project Description:

The 2-[^{14}C]deoxyglucose method affords a novel and unique opportunity to map functional neural pathways simultaneously in all anatomical components of the central nervous system. This method, therefore, allows the identification of complex neural circuits that are functionally active during various behavioral manipulations.

The goal of the present project is to map regions of the rat brain activated during rewarding electrical brain self-stimulation. The self-stimulation phenomenon, in which rats perform an operant task in order to receive brief trains of electrical pulses directly to various regions of their brains is recognized as an artificial activation of the brain's normal positive reinforcement mechanisms.

Previous work, in which rates of glucose utilization were measured in rats lever pressing to receive electrical stimulation to the ventral tegmental area (VTA), revealed a selective yet widespread pattern of metabolic activation in the terminal fields of the VTA including the prefrontal cortex, nucleus accumbens, amygdala, locus coeruleus, and lateral septum. In further experiments we have also examined self-stimulation to the substantia nigra pars compacta (SN), an area anatomically continuous to the VTA which contains dopaminergic cell bodies as does the VTA. The changes in metabolic activity associated with self-stimulation to the SN were widespread and bilateral in nature. In some cases the changes were similar to those seen with self-stimulation to the VTA.

The cells of the VTA and the SN despite a large number of common properties also have some divergent electrophysiological, neurochemical characteristics as well as different patterns of afferent and efferent anatomical connections. Because of the similarities in the patterns of changes in glucose utilization associated with self-stimulation to these two areas, we undertook a more comprehensive examination in which self-stimulation to the VTA and SN were compared behaviorally and metabolically. The purpose of these experiments was to answer the question, "Are the neural substrates underlying self-stimulation to the VTA and SN similar or different?"

The behavior of self-stimulating animals with electrodes in either the SN or VTA was compared in an observational study in which the presence or absence of a variety of behavioral classes was noted. Sniffing, rearing, turning, and exploratory behavior was most frequently observed during self-stimulation to the VTA, whereas stimulation induced motor artifacts and freezing were more commonly observed during SN self-stimulation. Behaviorally, therefore, self-stimulation to these two areas is different.

A direct comparison of the rates of glucose utilization associated with self-stimulation to the SN and VTA was carried out as well. In areas such as the hippocampus, olfactory tubercle, central amygdala and bed nucleus of the stria terminalis, metabolic rates in animals self-stimulating to the VTA were significantly higher than in animals self-stimulating to the SN. In contrast,

rates of glucose utilization in the anterior cingulate and in the caudate were significantly higher in animals self-stimulating to the SN, but not to the VTA. The pattern of functional activity associated with self-stimulation to the VTA is different from the pattern associated with self-stimulation to the SN. It can, therefore, be concluded that multiple neuronal systems underlie brain self-stimulation.

There were, however, a core set of structures in which metabolic rates were similarly increased for both groups in an equivalent fashion. These areas include the prefrontal cortex, nucleus accumbens, lateral septum and the mediodorsal nucleus of the thalamus. This convergence of metabolic activation suggests that these regions may be central to the production and maintenance of positively reinforced behavior.

Significance to Biomedical Research and to the Program of the Institute:

The 2-deoxyglucose method has allowed for the first time the simultaneous visualization of the functional neural circuits specific to the performance of self-stimulation behavior. Because self-stimulation is a basic model of positive reinforced behavior, elucidating the areas of the brain involved in this behavior can provide information about the neural systems involved in motivated and learned behavior. Further, psychiatric disease states such as depression have been ascribed to malfunction in brain reward systems. Studies of self-stimulation can provide useful information regarding the etiology of such disease states.

Proposed Course:

1. The application of the 2-deoxyglucose method to studies of reinforced behavior will be extended to include behavior reinforced by other reinforcers other than brain stimulation. Lever pressing for water will be studied to see if similar patterns of metabolic activity are obtained.

2. The administration of psychostimulants such as cocaine is considered highly rewarding. Studies will be extended to see if the effects of cocaine on cerebral metabolic activity are similar to those of brain stimulation.

Publications:

Porrino, L.J., Esposito, R.U., Seeger, T., and Crane, A.M.: Patterns of brain energy metabolism associated with rewarding brain stimulation of the substantia nigra. J. Cerebral Blood Flow Metab. (in press), 1985.

Esposito, R.U., Porrino, L.J., and Seeger, T.F.: Brain stimulation reward: measurement and mapping of effects by psychophysical techniques and quantitative 2-[^{14}C]deoxyglucose autoradiography. In Bozarth, M.A. (Ed.): Methods of Assessing the Reinforcing Properties of Abused Drugs. Brunswick, ME, Haer Institute, 1985, (in press).

Goldman-Rakic, P.S. and Porrino L.J.: The primate mediodorsal (MD) nucleus and its projection to the frontal lobe. J. Comp. Neurol. (in press), 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02217-02 LCM

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Plasticity in the Developing Monkey Visual System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Carolyn B. Smith	Chemist	LCM, NIMH
Others:	Louis Sokoloff	Chief	LCM, NIMH
	Susan Herdman	Guest Researcher	LCM, NIMH
	Kentaro Mori	Visiting Fellow	LCM, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.8	.30

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The postnatal development of the central visual pathways depends on the quality of the visual environment. During the critical period in the primate visual system environmental manipulation can modify the physiological properties of visual cortical cells. The purpose of this project is to study the underlying biochemical events that imbue the nervous system with the property of plasticity. Protein synthesis is a biochemical process which is involved in bringing about changes in morphology, adjustments in growth rates, and remodeling and maintenance of structures. We have therefore used the [^{14}C]leucine method to study the relationships between local plastic changes which occur in the developing monkey visual system and local rates of protein synthesis.

Project Description:

The purpose of this project is to study the biochemical events associated with plasticity. The developing rhesus monkey visual system has been chosen as a model system because the physiological and anatomical responses to deprivation have been so well described by others. We have focussed initially on the process of protein synthesis because it is a requirement for growth and development and because changes in morphology and rates of growth and remodeling and even maintenance of existing structures should be reflected in changes in rates of protein synthesis. Local rates of protein synthesis were determined with the [^{14}C]leucine method.

The postnatal development of the central visual pathways depends on the quality of the visual environment. During the critical period environmental manipulation can modify the physiological properties of visual cortical cells. If a monkey is monocularly deprived during the first few weeks of life there is a reorganization of the striate cortex such that the ocular dominance columns representing the functioning eye extend their boundaries and broaden at the expense of the adjacent columns representing the deprived eye. Eventually, most of the striate cortex may be incorporated into a monocular visual system that serves only the deprived eye. The organization of the lateral geniculates (dLGN), the locus of the cell bodies of the terminals in striate cortex, remains normal.

In early studies the effects of acute and chronic monocular deprivation in the newborn rhesus monkey on rates of protein synthesis in the laminae of the dLGN were examined. The results showed that in newborn monkeys with acute monocular deprivation produced no differential changes in rates of protein synthesis in any of the dLGN laminae. Chronic monocular deprivation resulted in decreases of about 15% in the rates of protein synthesis in the laminae innervated by the deprived eye whereas in geniculate laminae innervated by the functioning eye rates of protein synthesis were normal as compared with monkeys with binocular vision. These effects on protein synthesis occur when the deprivation is begun at a point in the critical period when there is considerable overlap of the representation from the two eyes in layer IV of striate cortex. Protein synthesis is most affected in the deprived dLGN cells. These results suggest that the underdevelopment of the deprived columns in striate cortex may be the result of inadequate growth and/or maintenance of axon terminals with consequent default of synaptic connections to the normally maintained terminals of the functional pathway.

In experiments in which monocular deprivation is begun later in the critical period when there is less overlap in striate cortex, the primary effect is on the nondeprived laminae of the dLGN. In these experiments the nondeprived laminae have increased rates of protein synthesis in comparison to normal, age-matched controls with binocular vision, and the deprived laminae are unchanged. Because there is little overlap, the reorganization that occurs as a result of the monocular deprivation may require a more active process than the default that was seen when monocular deprivation was begun at birth.

In order to determine if the changes in protein synthesis are the causes or the consequences of the reorganization of striate cortex, we have studied the effects of binocular deprivation. Physiological experiments have shown that binocular deprivation during the critical period has no effect on the organization of striate cortex. In our experiments on the effects of binocular deprivation during the first 25 days of life rates of protein synthesis were reduced by about 20% in all of the laminae of the dLGN. Although it is known from physiology experiments that the competitive advantage is not upset by binocular deprivation, this procedure may have influenced the extent of all of the terminal fields in the striate cortex.

The effects of deprivation on protein synthesis in striate cortex are also being examined in our experiments. In monkeys monocularly deprived for the second 25 days of life and in reverse-sutured monkeys, changes in the [^{14}C]leucine autoradiographic pattern in striate cortex can be seen. In both cases columns with alternating high and low rates of protein synthesis were evident. The columns were perpendicular to the cortical surface with a periodicity of about 0.8 mm. In the reverse-sutured monkeys the columns were particularly distinct in layers 2-4, while in the 25-50 day monocularly deprived animals columns in layer 4 were most prominent. These results show that in the newborn monkey the reorganization that occurs in response to chronic visual deprivation is reflected in changes in protein synthesis in the cells along the visual pathway.

Other experiments in progress are designed to further examine the processes involved in the plasticity demonstrated by this system. In addition, we have studied two prepubescent monkeys to test whether or not this protein synthesis response occurs in monkeys with fully developed visual systems. In both cases we found no significant effect.

Significance to Biomedical Research and Program of the Institute.

Plasticity, the capacity of the nervous system to respond to changes in the environment, is one of the most fundamental properties of nervous tissue. Learning, a form of plasticity, is a process of intense interest to neuroscientists the world over. In an attempt to study some of the biochemical processes underlying plastic changes, we have embarked on this study of the developing monkey visual system about which the physiology and anatomy are well-known. Studies with the [^{14}C]leucine method for local rates of protein synthesis and the [^{14}C]deoxyglucose method for local rates of glucose utilization are directed at first a description of some of the biochemical events which occur and then a determination of the regulation of these events. The understanding of these events may provide some insight into the unique properties of the critical period which make it so responsive to environmental manipulation. In addition, this research may have some direct implications on the clinical management of children with congenital cataracts and strabismic amblyopia.

Proposed Course.

Additional experiments on various forms of binocular and monocular deprivation have been carried out and autoradiographs are being analyzed. Future plans include experiments on the effects of monocular deprivation on monkeys with a split optic chiasm in order to further distinguish between the effects of deprivation per se and the effects of competition at the level of striate cortex on protein synthesis in the dLGN. Another approach to this question of competition vs. deprivation is the analysis of the monocular crescent area of the dLGN in experiments already completed.

With the addition of several older monkeys to our control animals at 0, 25 and 50 days of age, we have the beginning of a developmental time course of local rates of protein synthesis throughout the CNS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02220-02 LCM

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regional Biochemical Changes in the Normal Aging Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Carolyn B. Smith	Chemist	LCM, NIMH
Others:	Louis Sokoloff	Chief	LCM, NIMH
	Marian C. Diamond	Professor, Dept. of Physiology-Anatomy, Univ. of California	

COOPERATING UNITS *(if any)*

Department of Physiology-Anatomy, University of California, Berkeley, CA

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Developmental Neurochemistry Section

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.15	.10	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies are being carried out on the effects of aging on cerebral protein synthesis and glucose utilization in rats. With the application of local methods developed in this laboratory discrete regions of the brain can be examined in normal conscious animals. The regional changes in glucose utilization indicate that entire sensory pathways are affected by the aging process. The fact that similar changes are found in the same pathways with respect to protein synthesis suggests that some of these changes reflect an adaptation of the nervous system to a chronic lack of input. The basis of some of the changes which occur with age can be further examined in studies with pharmacological agents as well as in conjunction with behavioral measurements.

Project Description:

Aging is generally associated with a reduction in cerebral functional capacity. In view of the specificity of some senescent changes in human subjects the question of regional biochemical changes was examined in these studies on normal albino rats. Normal aging in the rat is also accompanied by some behavioral as well as histopathological changes in the brain. The autoradiographic deoxyglucose method was applied to examine the effects of normal aging on local cerebral glucose utilization. Decreases with age were found in the brain as a whole. On a local level senescent decreases were found with the most profound effects in the components of the primary auditory and visual pathways. These were effects similar to those seen following acute sensory deprivation of these systems. These results raised the question of whether or not some of the central nervous consequences of normal aging might not be due to sensory deprivation due to sense-organ degenerative changes inasmuch as there is known to be some retinal and inner ear degenerative change with age. With only a few exceptions the rates of glucose utilization in structures of the limbic and motor systems remained unchanged with age. The exceptions were several regions of white matter and the caudate-putamen.

The caudate-putamen which is part of the nigrostriatal dopaminergic system is a prominent site of changes during senescence. In order to further examine the functional consequences of these changes in the nigrostriatal system the deoxyglucose method was used to study the effects of normal aging on the metabolic responsiveness of dopamine-receptor activation by systemically-administered apomorphine. Significant dose-dependent effects of this dopamine-agonistic drug were found in 6 of the 14 brain regions examined in the young rats. In the lateral habenula and anterior cingulate cortex the effect of apomorphine was to decrease the rate of glucose utilization whereas in the subthalamic nucleus, inferior olivary nucleus, substantia nigra (pars compacta), and substantia nigra (pars reticulata) apomorphine stimulated glucose utilization. Age-dependent changes in responsiveness to apomorphine were found in the subthalamic nucleus, substantia nigra (pars reticulata), and inferior olivary nucleus. In the subthalamic nucleus the stimulation of glucose utilization by apomorphine was decreased in the old rats at all doses including those that elicited maximal responses. In the inferior olivary nucleus and the substantia nigra (pars reticulata) the dose-response curves were markedly depressed in the aged group. No significant effect of apomorphine on the rate of glucose utilization was found in the caudate-putamen as a whole, but a significant stimulation of glucose utilization was found only in the ventral portion in the young animals. The significant age-dependent decreases in responsiveness to apomorphine found in the subthalamic nucleus, substantia nigra (reticulata) and inferior olivary nucleus may reflect the functional consequences of the reported loss of dopamine receptors in the caudate-putamen with aging.

Measurements of energy metabolism do not differentiate between the immediate functional demands of cerebral structures and the longer term maintenance processes within the nervous system. Long term effects that are related to changes in morphology, structural maintenance, and remodeling in the nervous system are more likely reflected in biosynthetic biochemical processes,

such as protein synthesis. In another study the effects of aging on local rates of protein synthesis in brain were examined by means of the quantitative autoradiographic [^{14}C]leucine method. The results show that aging is associated with significant decreases in rates of protein synthesis in the brain as a whole as well as in several specific brain regions. Brain regions involved in visual and auditory function were selectively affected, perhaps due to a chronic lack of sensory input. Several regions involved in motor function and 2 areas in the limbic system had significantly decreased rates of protein synthesis in the old rats. Notably, there was a significant age-related decrease in protein synthesis in the locus coeruleus which contains the cell bodies of origin of the major ascending noradrenergic innervation of the cortex.

The work of Rosensweig, Diamond and others has shown that environmental enrichment can result in histological changes in the cerebral cortex of adult rats. As environmental enrichment may be the converse of sensory deprivation, we are examining the effects of enrichment on local rates of cerebral glucose utilization. These experiments have been carried out in collaboration with Dr. M. Diamond. Twenty four Long-Evans male rats were divided into two groups: 1) standard colony conditions i.e., 3 rats to a standard cage and 2) enriched conditions, i.e., 12 rats to a large cage containing objects with which the animals can interact. Determinations of local rates of cerebral glucose utilization were carried out on 6 rats from each group, and measurements of cortical thickness were made on 6 rats from each group. These results are being analyzed.

Significance to Biomedical Research and Program of the Institute.

Insofar as aging is rapidly becoming a problem of increasing social significance this research which is focused on senescent changes in the ability of the brain to function may be of considerable importance to the medical community. Furthermore, our results indicate that some of the changes that occur with age may be the consequence of a decreased functional activity. Confirmation of this possibility and further understanding of the basic biochemical processes underlying plastic changes in the nervous system of either an involutional or developmental nature may be useful in trying to prevent and/or reverse such senescent changes.

Proposed Course.

Completed experiments on the effects of aging on protein synthesis and glucose utilization will be further analyzed. In particular, the nucleus basalis in which there are known pathological changes in Alzheimer's Disease will be examined. In addition the results of experiments of environmental enrichment are being analyzed.

Publications:

Smith, C.B.: A comparison of the effects of aging on local rates of cerebral glucose utilization and protein synthesis in Sprague-Dawley rats. In In Lassen, N.A., Hossmann, K.A., Reivich, M., Agnoli, A., and Cahn, J. (Eds.): Drugs and Diseases. London, England, John Libbey Eurotext Limited, 1984, Vol. 1, No. 3, pp. 75-84.

Smith, C.B.: Effects of aging on local rates of cerebral energy metabolism and protein synthesis in Sprague Dawley rats. In Depresseux, J.C. (Ed.): Circulation et Metabolisme du Cerveau. Montrouge, France, John Libbey Eurotext Limited, 1985, Vol. 2, Supplement 1, pp. 58-75.

Sokoloff, L.: The aging brain: Introduction. In Depresseux, J.C. (Ed.): Circulation et Metabolisme du Cerveau. Montrouge, France, John Libbey Eurotext Limited, 1985, Vol. 2, Supplement 1, pp. 9-14.

Ingvar, M.C., Maeder, P., Sokoloff, L., and Smith, C.B.: Effects of ageing on local rates of cerebral protein synthesis in Sprague-Dawley rats. Brain 108: 155-170, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00900-29 LCM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Studies on Myelin and Myelin Basic Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. E. Martenson	Research Chemist	LCM, NIMH
Others:	G. E. Deibler	Chemist	LCM, NIMH
	M. L. Pedersen	Biologist	LCM, NIMH
	A. Stone	Research Chemist	LCB, NIMH
	E. C. Alvord, Jr.	Professor, Univ. of Wash. Sch. of Med.	
	G. Mendz	Assoc. Prof., University of Sydney	
	N. Sternberger	Assoc. Prof., University of Rochester	

COOPERATING UNITS (if any)

Neuropathology Dept., University of Washington Sch. of Med., Seattle, Washington
School of Chemistry, University of Sydney, New South Wales, Australia
Center for Brain Research, University of Rochester, Rochester, New York

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are continuing to pursue our studies on the conformation of the myelin basic protein (BP) by 1) circular dichroism spectroscopy, 2) immunological reactions, 3) chemical cross-linking, and 4) nuclear magnetic resonance spectroscopy. In addition, we are 5) isolating peptic fragments of the BP which contain in vivo phosphorylated serine and threonine residues for use in preparing antibodies specific to the phosphorylation sites.

Project Description:

Objectives:

The major objective of the current work has been to elucidate the three-dimensional structure of the myelin basic protein (BP) under a variety of solution conditions and when bound to amphipathic molecules in an attempt to understand the function of the protein in the myelin sheath.

Methods Employed:

The work has involved nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopy and immunological reactions of the BP and specific fragments thereof from a number of species as well as the preparation of large quantities of the BP fragments useful in such studies.

1) In a collaboration with Dr. Audrey Stone, various fragments of the BP have been examined for their "conformational adaptability" in water-trifluroethanol (TFE) solutions. This was done to determine the types (α-helix and β-sheet) and locations of secondary structures which the BP could assume under conditions in which intramolecular hydrogen bonding is favored. This bonding will lead to a more compact structure resembling that which is believed to exist in situ.

The first study was carried out on the intact rabbit BP and on peptides 1-95 and 96-168 produced from the BP by thrombic cleavage. Computer analyses of the circular dichroism spectra showed that, contrary to popular belief, the BP contains β-structure (~30 residues) in dilute aqueous solution at neutral pH. No α-helix is present. Each of the peptides (1-95 and 96-168) contained ~17 residues in β-structure under these conditions. Each responded differently to increasing concentrations of TFE. In peptide 1-95, ~20 residues became helical by 30% TFE, whereas in peptide 96-168 only ~7 residues adopted the α-helical conformation. At higher TFE concentrations (up to 92%), peptide 1-95 showed two additional α-helix transitions, one between 40 and 75% TFE and the other above 75% TFE. These resulted, respectively, in the incorporation of ~10 and ~20 additional residues into α-helical structures. Peptide 96-168, on the other hand, underwent only one additional transition to α-helix (above 75% TFE), which accounted for ~20 residues. In general, increasing TFE concentration up to 75% increased the amount of β-structure in each peptide to ~25 residues. In peptide 1-95, however, a slight decrease in β-structure occurred at ~20% TFE, concomitant with the sharp rise in α-helix content of this peptide. Above 75% TFE a marked reduction in β-structure content (to ~5 residues) occurred in each peptide, which resulted from β → α transitions.

On the basis of hydropathy considerations and secondary structure predictions, it was possible to provide a tentative assignment of β-structure- and α-helix-forming regions to specific amino acid sequences of the protein. The β-structure observed in the intact BP in the absence of TFE could be ascribed to β-sheet formation resulting from the association of the 5 most hydrophobic sequences of the BP: residues 15-21, 37-45, 84-92, 106-112, and 148-154. In

peptide 1-95 the initial 3-stranded sheet could break down into a 2-stranded sheet by a $\beta \rightarrow \alpha$ transition of sequence 15-21, leading to a long α-helix of residues 7-29 at 30% TFE. The remaining 2-stranded sheet 37-45/84-92 could elongate slightly under these conditions. Between 40 and 75% TFE additional, short helices could form in sequences 58-63 and 70-75, corresponding to the second helix transition. Above 75% TFE essentially all of the 2-stranded β-sheet could be converted to α-helix. In peptide 96-168 the initial 2-stranded β-sheet 106-112/148-154 could elongate slightly up to 75% TFE, then undergo a $\beta \rightarrow \alpha$ transition at higher TFE concentrations. The α-helix that formed at 30% TFE could be assigned to sequence 129-140.

Identical studies were carried out on thrombic peptides 1-63, 64-95, 96-128, and 129-168 to check these tentative assignments of α-helix- and β-structure-forming regions and to see if the behavior of the larger peptides (1-95 and 96-168) could be interpreted in terms of the behaviors of their respective parts. While largely confirming the assignments, these results also showed that the secondary structures observed in the larger peptides resulted to some extent from interactions between non-contiguous portions of their polypeptide chains as well as from the independent contributions of the smaller peptides. It is therefore to be expected that similar interactions between non-contiguous portions of the intact BP will produce a pattern of secondary structure changes in TFE somewhat different from those observed with peptides 1-95 and 96-168. Preliminary results indicate that the intact protein has a greater tendency to form α-helices than does its peptides and that it more readily undergoes $\beta \rightarrow \alpha$ transitions.

2) In a collaborative study with Dr. E. C. Alvord, Jr., the specificities of two monoclonal antibodies (MAbs) evoked in mice with guinea pig BP have been examined and interpreted in terms of a specific folding of the protein's polypeptide chain. Studies with guinea pig and rabbit BP fragments showed that a region encompassing the central Phe-Phe sequence is obligatory, but not sufficient, for reactivity with the first MAb. Appreciable reactivity was observed for rabbit peptides 22-95 and 45-151, and lower, but significant, reactivity was shown by peptide 32-95. Only very weak reactivity was seen with peptide 44-95. These results suggest that the epitope to this MAb is formed by specific polypeptide chain folding that requires participation of the hydrophobic sequence Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val (84-92). We propose that this sequence can be stabilized in a specific conformation by antiparallel β-sheet formation with sequence 37-45 and/or sequence 106-112. At one extreme, sequence 84-92 in β-structure could constitute the major part of the epitope; at the other extreme, it could simply organize the remainder of the protein into a conformation that is recognized by the MAb. The second MAb reacts only with guinea pig BP and fragments containing the species-restricted sequence Arg-Ala-Asp-Tyr-Lys-Ser-Lys (129-135). The smallest available fragment that displayed full reactivity was peptide 22-164; peptides 45-167, 89-167, and 119-167, on the other hand, were only about one-fourth as reactive as the intact protein. These results suggest that sequence 22-44 constitutes a region in peptide 22-164 or in the intact protein which interacts with sequence 129-135 and stabilizes the latter in the appropriate antibody-binding conformation.

3) Dr. Clara Monferrán has been engaged in studies aimed at examining the manner in which the polypeptide chain of the BP folds in three dimensions, as well as the sites involved in BP-BP interactions. We consider these studies to be important because dimerization of the BP across the cytoplasmic surfaces of the myelin lamellae could be a mechanism of myelin compaction. She has been introducing intra- and inter-molecular cross-links at lysine residues with bifunctional amidoesters, which, after digestion of the cross-linked products by proteases and isolation of the cross-linked fragments, can be cleaved to show which parts of the polypeptide chains were in close proximity when the cross-linking reactions occurred. She has determined the optimal conditions for cross-linking in the presence of detergents (sodium dodecyl sulfate, Triton X-100, and lysolecithin) and is in the process of determining the specific residues that are cross-linked in the monomeric and dimeric forms of the protein.

4) Studies have continued in collaboration with Dr. George Mendz to assign all of the histidine resonances in the BP and to determine the conformation of the antigenic site within residues 1-14. The latter NMR studies have shown that interactions occur between Ala-1 and Gly-11 and between His-10 and Tyr-14, indicating a good deal of folding within the peptide.

5) Large amounts of BP have been digested with pepsin to produce sufficient amounts of several large peptides whose phosphorylated species are being isolated and purified. Dr. Nancy Sternberger will try to prepare monoclonal antibodies against the phosphorylated regions of the peptides for immunohistochemical studies. Such antibodies should also be extremely useful in isolating the phosphorylated species of BP for metabolic and physiochemical studies of the protein.

Significance to Biomedical Research and the Program of the Institute

Knowledge gained from the studies described above will help us to understand the assembly and maintenance of the myelin sheath and how the BP contributes to these processes. In addition, it will provide insight into the nature of some of the pathological processes involving loss of myelin, in particular multiple sclerosis.

Proposed Course:

These studies of myelin basic protein are being discontinued because they have been considered irrelevant to the mission of the NIMH.

Publications

Nygaard, E., Mendz, G.L., Moore, W.J., and Martenson, R.E.: N.M.R. of a peptic peptide spanning the triprolyl sequence in myelin basic protein. Biochemistry 23: 4003-4010, 1984.

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Kira, J., Deibler, G.E., Krutzsch, H.C., and Martenson, R.E.: Amino acid sequence of porcine myelin basic protein. J. Neurochem. 44: 134-142, 1985.

Hruby, S., Alvord, E.C., Jr., Martenson, R.E., Deibler, G.E., Hickey, W.F., and Gonatas, N.K.: Sites in myelin basic protein that react with monoclonal antibodies. J. Neurochem. 44: 637-650, 1985.

Law, M.J., Deibler, G.E., Martenson, R.E., and Krutzsch, H.C.: Cleavage of rabbit myelin basic protein by plasmin. Isolation and identification of the major products. J. Neurochem., in press, 1985.

Stone, A.L., Park, J.Y., and Martenson, R.E.: Low ultraviolet circular dichroism spectroscopy of oligopeptides 1-95 and 96-168 derived from myelin basic protein of the rabbit. Biochemistry, in press, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00901-30 LCM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Reactivity of Myelin Basic Protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. W. Kies	Chemist	LCM, NIMH
Others:	B. F. Driscoll	Research Biologist	LCM, NIMH
	J. Kira	Visiting Vellow	LCM, NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Immune mechanisms in the induction of EAE have been studied in two species: Str 13 guinea pigs and outbred rabbits. a) Chronic (demyelinating) EAE can be induced in Str 13 guinea pigs by a combination of BP-sensitized cell transfer and sensitization of the recipients with whole CNS tissue (guinea pig cord). Chicken brain (which is non-encephalitogenic) can be substituted for guinea pig cord (which contains encephalitogenic BP). Fractionation of the former to identify the "antigen" responsible for inducing demyelination has indicated localization of the "antigen" in myelin. Further fractionation has been unsuccessful.
b) The specificity of the rabbit encephalitogenic site has been investigated with purified proteolytic fragments of BP. The first site reported to be active was located in peptic fragment 45-87. We and others noted that Res 1-44 were also active. We have further localized that site to Res 15-31, with overlapping peptides. Most assays of individual fragments failed to elicit 100% response in a group of rabbits. We questioned whether this was caused by genetic differences in the individual rabbits. Eighteen rabbits were sensitized with whole BP and the proliferative response of their lymph node cells was tested. Some rabbits responded only to Fragment 45-87; some to both (45-87) and (1-44). In general, response to both BP and fragments was higher when clinical signs of EAE were positive. The site in Fragment 45-87 appeared to be the most active.

Project Description:

Objectives:

The broad objective of this project is to study immune mechanisms in the pathogenesis of EAE. This year two separate studies were carried out - a) An analysis of the CNS constituent which induces chronic EAE in animals that have received a suboptimal transfer of BP-sensitized cells, and b) Identification of the rabbit encephalitogenic site in the N-terminal 40 residues of myelin basic protein and correlation of the in vitro proliferative response of BP-sensitized T cells to the encephalitogenic fragment with the ability of the fragments to induce EAE.

Methods Employed:

a) Transfer of BP-sensitized Str 13 cells to naive Str 13 recipients and sensitization of cell recipients with (non-encephalitogenic) chicken brain or fractions.

b) Sensitization of rabbits with myelin basic protein and isolated proteolytic fragments - assay of their encephalitogenic activity and their ability to induce an in vitro proliferative response.

Major Findings:

a) It has been reported (Driscoll et al.) that chronic EAE (demyelination) can be induced in Str 13 guinea pigs by a combination of active and passive sensitization - a suboptimal transfer of BP-sensitized cells followed by sensitization of the recipient with whole CNS tissue. Because the BP in chicken brain is non-encephalitogenic, we have substituted chicken brain for the non-encephalitogenic portion of guinea pig cord and injected BP simultaneously in a separate site. We had previously determined that this provided a sensitization comparable to that of whole guinea pig cord in its ability to induce demyelination. BP alone is ineffective but BP plus an auxiliary antigen induces demyelination. Attempts to fractionate and identify the factor in chicken brain responsible for induction of chronic demyelinating EAE have been partially successful. The active material has been localized in myelin, but further fractionation has either resulted in complete loss of activity or distribution of reduced activity in all fractions. Several fractionation schemes have been explored unsuccessfully. Furthermore, attempts to induce demyelination by incorporating BP into liposomes have also produced negative results.

b) We reported previously that the N-terminal peptide fragment of BP (Res 1-44) appeared to be more active in rabbits than the adjacent peptic peptide (Res 45-88), which had been found to be active. We have localized the activity in the N-terminal fragment to Res 15-31 by assay of overlapping fragments (Res 15-44 and 1-31 are active, whereas 1-14 is inactive). Considerable variability was noted in the disease response of our experimental rabbits to various fragments of BP. Only 50-75% of an experimental group became ill after sensitization with a given fragment. It appeared that this might be the result

of individual rabbits reacting to different sites in the molecule. We examined this variability of response by sensitizing rabbits with whole BP and determining the proliferative response of their cells to individual fragments. If, indeed, individual rabbits were responding to different sites the proliferative responses should have shown this. Our prediction was that genetic differences would result in the cells reacting to one or the other fragment. The cellular response was primarily to Peptide 45-87, rather than to Peptide 1-44, but the results did not show individual rabbits reacting to one or the other. There were two distinct groups of rabbits. Cells of one group responded to peptide 45-87 only, and cells of the second group responded to both of the N-terminal peptides, 1-44 and 45-87. The proliferative response was, in general, more pronounced in rabbits developing clinical signs of EAE than in rabbits not developing clinical signs.

Significance to Biomedical Research and Program of the Institute:

It has been thought for many years that multiple sclerosis may be an autoimmune disease. More recently, it has been suggested that Alzheimer's may also be the result of an adverse autoimmune reaction. We have investigated the mechanism of autoimmune damage to the CNS in a controlled experimental situation in order to provide a theoretical basis for treatment and/or prevention of these and other CNS diseases. Inasmuch as the specificity requirements for human autoimmune responses are unknown, it is important to extend our knowledge of the species-specificity of the only known CNS immunogen (BP) to as many species as possible.

Proposed Course:

No further work is planned for this project.

Publications

Kies, M.W.: Experimental Allergic Encephalomyelitis. In Lajtha, A. (Ed.). Handbook of Neurochemistry, Vol. 9. New York, Plenum Publishing Corporation, 1985, pp. 533-552.

Kies, M.W.: Myelin Basic Protein (BP) - A Short Review. In Adelman, G. (Ed). Encyclopedia of Neuroscience. Massachusetts, Birkhauser Boston, Inc., 1985, in press.

Kies, M.W.: Experimental Allergic Encephalomyelitis. In Adelman, G. (Ed). Encyclopedia of Neuroscience. Massachusetts, Birkhauser Boston, Inc., 1985, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00902-20 LCM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. F. Driscoll	Research Biologist	LCM, NIMH
Others:	M. W. Kies	Chemist	LCM, NIMH
	E. C. Alvord, Jr.	Professor, Univ. of Washington School of Med.	

COOPERATING UNITS *(if any)*

Neuropathology Department, University of Washington School of Medicine, Seattle, Washington

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To analyze the cellular mechanisms responsible for the central nervous system (CNS) autoimmune disease, experimental allergic encephalomyelitis (EAE), factors responsible for activating cells which transfer this disease are examined in vitro. One factor of critical importance is the lymphokine interleukin 2 (IL-2) which regulates proliferation of T-lymphocytes. However, exposure of BP-specific cells to IL-2 alone is not sufficient to insure efficient transfer of EAE; if recipients are also injected with antigen non-specific cells producing the monokine interleukin 1 (IL-1), recipients develop severe EAE. Based on data from in vitro experiments, it appears that the IL-1 producing cells interact with the IL-2-activated, BP-specific cells in vivo. This interaction is required if the proliferating BP-specific cells are to function maximally in vivo and induce severe clinical and histologic EAE.

In addition to the inflammatory reaction in the CNS induced by BP-sensitization in acute EAE, demyelination can also be induced in a chronic form of EAE. Our data clearly indicate that the antigen(s) responsible for this demyelination are found in the myelin-fraction of CNS tissue and are separate from the encephalitogenic BP. However, concurrent sensitization with BP and the antigens responsible for demyelination is required to induce in vivo demyelinating lesions in the CNS.

Project Description:

Objectives:

To understand the step-by-step mechanisms by which antigen-specific cells can infiltrate the CNS and induce inflammatory lesions responsible for the pathogenesis of acute EAE and to identify the CNS antigens responsible for the demyelination that is observed in chronic, relapsing EAE.

Methods:

BP-sensitized T-lymphocytes from Lewis rats are exposed in vitro to soluble factors (lymphokines) and transferred to naive recipients to assess the effect of the soluble factors on the transfer of acute EAE. Chronic demyelinating EAE is assessed in strain 13 guinea pigs sensitized with various non-encephalitogenic antigens following transfer of BP-sensitized cells to determine the antigen responsible for CNS demyelination which accompanies the CNS inflammation in this model of human multiple sclerosis (MS).

Major Findings:

In transfer experiments, the BP-specific cells responsible for EAE proliferate in response to IL-2 in vitro but these cells fail to transfer EAE when injected in vivo. If the recipients are also injected with IL-1 producing (antigen non-specific) cells, the recipients develop severe EAE. The IL-1 producing cells (macrophages?) probably interact directly with the IL-2 activated, BP-specific cells in vivo and "organize" the cells so that proliferation and development can continue. The BP-specific cells then induce CNS lesions. In chronic EAE, antibodies to myelin constituents, enter the acute CNS lesions (via the damaged blood-CNS barrier) and attach to the myelin leading ultimately to its phagocytosis. The identity of the antigen(s) in myelin responsible for the demyelinating antibodies has not been clarified but they are separate from the encephalitogenic BP.

Cells involved in CNS inflammation - BP-specific lymphocytes cultured in the presence of BP transfer EAE to naive recipients in a greatly enhanced fashion. The system is relatively unique in immunology since it involves both an in vitro phase and an in vivo phase. Therefore, successful transfer depends not only on activation of the appropriate pathways in vitro, but the subsequent in vivo survival, migration and development of the in vitro activated cells. From our data, it appears that in vitro exposure of the BP-specific cells to IL-2 is of critical importance. Exposure to IL-2 also takes place in the normal course of events when cells are cultured with BP. However, in contrast to cells cultured with BP, cells exposed in vitro only to IL-2 do not efficiently transfer EAE to naive recipients. If these recipients are also injected with antigen-non-specific cells cultured with LPS they develop severe EAE. Thus while IL-2 fulfills the requirements for in vitro activation of cells, these cells do not function as well in vivo as do the cells cultured with BP.

Experiments were designed to test whether an in vitro assay system could effectively detect the synergistic interaction between the IL-2 activated BP-

specific cells and the LPS-activated cells that clearly takes place in vivo. The IL-2-activated, BP-specific cells and the LPS-activated cells are cultured together in microtiter plates in a criss-cross pattern covering three orders of magnitude of cell concentrations (2×10^6 of each cell/ml to 2×10^3 of each cell/ml). The data show that at moderate cell concentrations (comparable to the numbers of cells present in vivo?) there was a dramatic synergistic interaction between the two cell types as assessed by cell proliferation and obvious cell clustering in the wells. Based on these data, it appears that the LPS-activated cells "organize" the IL-2-activated cells in vivo, such that a local environment around these clusters contain high levels of the lymphokines these cells need to develop into EAE effector cells. When the IL-2 activated cells are injected without the LPS-activated cells, their migration to widely separate areas of the lymphoid tissue may deprive them of the necessary high concentrations of lymphokines required for their development and/or survival.

Presumably, when cells are cultured with BP, not only IL-2 but also factors such as macrophage activating factor (MAF), IL-1 and gamma interferon (γINF) are produced which activate the appropriate cells that then "organize" the IL-2-activated, antigen specific T-lymphocytes in vivo.

Chronic EAE - In addition to the inflammatory damage seen in acute EAE is the demyelination seen in chronic EAE. This demyelination is a separate component of the disease, at least in guinea pigs, and our data clearly indicate that the antigens responsible for the demyelination are separate from but dependent on the encephalitogenic activity of myelin BP. Recently, in collaboration with Dr. Dennis Bourdette in Dr. Fritz Seil's laboratory, we demonstrated a direct correlation between the occurrence of in vivo demyelination in chronic EAE animals and presence of factors in their sera capable of inhibiting the myelination of CNS tissue explants in vitro. This strengthens the hypothesis that factors demonstrated in vitro are responsible for the demyelination observed in vivo in guinea pigs with chronic EAE.

Significance to Biomedical Research and the Program of the Institute

An increasing number of human diseases of previously unknown etiology are being shown to have an autoimmune component which is responsible for some or all of the pathologic sequelae. Some diseases are mediated by autoimmune antibody production while others are caused by induction of sensitized cells. Our current knowledge of how the immune system is controlled in vivo is sparse. Most information is based on completely in vitro techniques and it is evident that treatments based on extrapolation from in vitro data to in vivo clinical situations have been disappointing. There is a clear and pressing need for immunological assays that are capable of functioning in vivo. EAE is the oldest such system and by far the best characterized. More work on this or similar in vivo systems will be required before intelligent intervention in human autoimmune diseases is possible.

Proposed Course:

Most work on this project is being terminated at the present time.

Publications:

Driscoll, B.F., Kies, M.W., and Alvord, E.C., Jr.: The nature of the defect in experimental allergic encephalomyelitis (EAE)-resistant Lewis (Le-R) rats. J. Immunol. 134: 1547-1570, 1985.

Woyciechowska, J., Goldstein, A.L., and Driscoll, B.: Experimental allergic encephalomyelitis in guinea pigs. Influence of thymosin fraction V on the disease. J. Neuroimmunol. 7: 215-219, 1985.

Driscoll, B.F., Kira, J., Kies, M.W., and Alvord, E.C., Jr.: Mechanism of demyelination in the guinea pig: Separate sensitization with encephalitogenic myelin basic protein and non-encephalitogenic brain components. Neurochem. Pathol., in press, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00903-08 LCM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of Degradation Products Associated with Human Myelin Basic Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. E. Deibler	Chemist	LCM, NIMH
Others:	M. W. Kies	Chemist	LCM, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cerebral Metabolism

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Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Further investigation of human myelin basic protein (HBP) extracted from whole CNS tissue or from CNS myelin, has confirmed the existence of two and possibly three HBP-related polypeptides, approximately 1.5 K smaller than the 18.5 K major HBP. These polypeptides differ in charge from the major 18.5 K HBP, component 1, by two negative charges. This difference is caused by the deletion of either the first 17 N-terminal residues, or 19 residues from the C-terminal part of the molecule. Tryptic digestion of the thrombic peptides of HBP (fraction 3), which is a 50/50 mix of 18.5 and 17.0 K polypeptides, gave indication that one of the 17 K polypeptides had a deletion immediately N-Terminal to the tryptophan. We have developed a new procedure on the FPLC (fast protein liquid chromatography) system to separate the 17 K polypeptides from the 18.5 K HBP, component 3. In collaboration with Dr. Henry Krutzsch, NCI, we have shown that both the 17 K and the 18.5 K have blocked N-terminals and the same C terminus. Our current tryptic peptide mapping by HPLC allows us to identify all of the tryptic peptides of HBP. Therefore, we can eventually identify all the deletions in the 17 K protein, which probably arise from m-RNA processing rather than autolysis in situ.

We are also investigating the relation of phosphorylation to the conformation of bovine myelin basic protein, in collaboration with Dr. Audrey Stone. Circular dichroic studies show that the percent of ordered structure (β-turns plus β structure) increases up to 46% with increased phosphate on the BP molecule. This indicates that BP has a significant tendency to form ordered structures in aqueous solution and suggests that BP phosphorylation may play a role in the way basic protein fits into the myelin in situ.

Project Description:

Objectives:

The elucidation of the sequences of all the 17 K polypeptides associated with human BP. Purification of a mono-phosphorylated bovine myelin basic protein for structural studies.

Methods Employed:

The new procedure for the preparation of human myelin basic protein, our very sensitive 15% PAGE system, and tryptic mapping on HPLC described in previous annual reports, have been used in this study.

When the tryptic digest of whole HBP (component 1) was subjected to a modified HPLC procedure, most of the peptides were separated cleanly. The peaks that were incompletely separated could nevertheless be identified by amino acid analysis. This technique will be used to identify the deletions in the 17 K polypeptides.

We have developed a new procedure on the FPLC for separating 17 K and the 18.5 K polypeptides in HBP (fraction 3). Previous attempts to purify the 17 K forms had failed. This purification method can be completed in 30 minutes at room temperature.

Major Findings:

Separation of the 17 K and 18.5 K forms of HBP (fraction 3) on FPLC permitted us to determine that the N-terminal residue of both proteins is blocked and that both proteins have the same C terminus. The amino acid composition of the 17 K polypeptide showed that 2 or 3 Ser, 1 Lys, 2 Gly, 1 Leu and 1 Phe are missing. The 18.5 polypeptide which was isolated with the 17 K form in Fraction 3, has the same composition as HBP (component 1) except that it contains 0.36 nmol of phosphoserine and 0.29 nmol of phosphothreonine per mg of protein. Tryptic peptide mapping with the modified HPLC program, will be used to identify the missing or modified portion of the 17 K polypeptide.

Preliminary data suggest that phosphorylation of myelin basic protein causes it to assume a more ordered structure in aqueous solution - up to 46% β-turn plus β-structure.

The isolation procedure of tryptic peptides by HPLC was used to obtain the tryptic peptides (74-78) from bovine, monkey, and rabbit peptic peptides (43-88) of the respective myelin basic proteins. In collaboration with Dr. Henry Krutzsch, we determined that the published order of residues (78-79) had been incorrectly assigned in all three species of myelin basic protein. The correct order is His-Gly. A similar analysis of tryptic peptide (80-91) of human basic protein confirmed the sequence Thr-Gln-Asp-Glu-Asn-Pro for residues 80-85.

Significance to Biomedical Research and the Program of the Institute

A normal functioning nervous system is impossible without myelin. In order for the neuron to process and transfer information, the axon must be capable of conduction, which is facilitated by myelin. In undertaking these studies on the structure and conformation of myelin basic protein, we believed that they would shed light on the structure of myelin. Whether aberrations or changes in the structure of myelin could affect its susceptibility to damage (demyelination) is not known, but it is reasonable to investigate such a possibility.

Proposed Course:

The isolation of the 17 K forms of human BP and the isolation of monophosphorylated bovine BP are partially completed. We would like to be able to finish the analytical studies on each preparation because they are important in the field of myelin chemistry and they involve collaboration with other NIH scientists. After they have been carried out and the results published, the project could be discontinued.

Publications

Deibler, G.E., Boyd, L.F., and Kies, M.W.: Enzymatic and nonenzymatic degradation of myelin basic protein. Neurochem. Res. 9: 1371-1385, 1984.

Deibler, G.E., Krutzsch, H.C., and Martenson, R.E.: A reinvestigation of the amino acid sequences of bovine, rabbit, monkey, and human myelin basic proteins. J. Biol. Chem. 260: 472-474, 1985.

Deibler, G.E., Boyd, L.F., Martenson, R.E., and Kies, M.W.: Isolation of tryptic peptides of myelin basic protein by reversed-phase high-performance liquid chromatography. J. Chrom. 326: 433-442, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00507-03 LCM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Brain Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert M. Cohen, M.D., Ph.D., Chief, CBI, LCM, NIMH

COOPERATING UNITS *(if any)*

Neuroscience Branch, NIMH; Biological Psychiatry Branch, NIMH; Clinical Center, NIH; Nuclear Medicine, CC, NIH; LPP, NIMH; Neuropsychiatry Branch NIMH, SEH: U. Calif. at Irving; Vanderbilt Med. Sch.; British Columbia Med. Sch.

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Clinical Brain Imaging

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The National Institute of Mental Health,
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.8	4.9	2.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major effort of this section continues to be the refining of existing methodologies for the study of cortical functioning based on positron emission tomography (PET) and to a lesser degree electrical brain mapping procedures particularly as they complement PET information. We continue to contribute to the development and comparison of three methods of data abstraction, the "peel", the "ring" and the region of interest (ROI). We have been examining the benefits of standardizing the abstracted PET data to emphasize that information that directly pertains to the activity of localized regions of the brain as opposed to the overall glucose metabolic rate (GMR) of the brain. To accomplish this we have explored multivariate analytical approaches other than that which has been previously used (repeated measures analysis of variance) which we feel is not suitable to the important biomedical questions that PET should be addressing.

We have pursued as thorough an analysis as possible of the FDG PET representations of two behavioral paradigms, repeated somatosensory stimuli and an auditory continuous performance test. They complement each other in that in the former the "normal" response is to habituate to a "benign" sensory stimulus, whereas the latter task requires selective attention. Differences are observable and suggest that PET is a modality where specific functionally distinct regions of the frontal cortex can be examined. These paradigms are currently in use to study normal habituation and selective attention and to evaluate whether these fundamental processes are altered in mental illness. To date, schizophrenic patients do not show the diminution in electrical response to repetitive somatosensory stimuli observed in normals. These differences appear to be reflected in a different PET pattern of GMR.

PHS 6040 (Rev. 1/84) GPO 904-917

OTHER PROFESSIONAL PERSONNEL

Lynn DeLisi, M.D., Staff Psychiatrist, BPB, NIMH
Henry H. Holcomb, M.D., Clinical Associate, LCM, NIMH
Richard Coppola, D.Sc., LPP, NIMH
Monte Buchsbaum, M.D., Department of Psychiatry, U. of California at Irving
David Pickar, M.D., NSB, NIMH
Robert M. Post, M.D., Chief, BPB, NIMH
Thomas W. Uhde, M.D., BPB, NIMH
Robert M. Kessler, M.D., Nuclear Medicine, Vanderbilt Medical School
Steven M. Larson, M.D., Chief, NM, CC, NIH
Campbell Clark, M.D., British Columbia (Medical School of)
Richard Margolin, M.D., NM, CC, NIH
John I. Nurnberger, M.D., British Columbia (Medical School of)
John Morihisa, M.D., NPB, NIMH at St. Elizabeths Hosp.
Daniel R. Weinberger, M.D., ATB, St. Elizabeths Hosp.

OBJECTIVE

The goals of this project are to develop and apply methods for imaging the brain based on its functional characteristics so as to further our understanding of normal and abnormal human behavior.

METHODS EMPLOYED

Behavioral Assessment

A detailed assessment and screening of all normal volunteers and patients participating in projects in the section occurs. This includes a structured interview, the Cannon-Spoor social adjustment scale, a detailed alcohol and drug history, family history and medical and psychiatric histories. Where appropriate, the Hamilton and Beck Depression scales, BPRS, the Strauss/Carpenter outcome scale, the global assessment scale, the AIMS (for motor movement ratings), and Krawiecka scale, to rate positive and negative symptom clusters in schizophrenia, are used. All subjects rate themselves using the Spielberger Anxiety Scale to report their experience during actual imaging procedures.

Biological Assessment

In conjunction with other laboratories subjects are often assessed for dexamethasone suppressibility, TRH responsiveness, serotonin platelet uptake, and drug treatment responsiveness. In addition, blood, urine and cerebrospinal fluid measurements reflecting neurochemical activity are in the future expected to be utilized in conjunction with electrophysiologic and PET data.

X-ray transmission tomography (CT Scan) is used for measurements of ventricular size, sulcal atrophy and hemispheric asymmetry. Positron Emission Tomography (PET) is also used. PET, utilizing similar reconstruction mathematics as that of CT scanning, enables the user to obtain slice images of radioisotope

cortical location. Using F^{18}-2-deoxyglucose (FDG) as the radioisotope tracer and the methods developed by Sokoloff and others, it is possible to obtain data on local glucose metabolism and consequently, probable local cortical functional activity.

Mapping of Electrical Activity

If a large number of electrodes are used, the recordings of EEG and evoked potential can also be used to develop images or maps of cortical activity, albeit of surface topology. Presently, 12 standard 10/20 system points on the left hemisphere and midline, and four additional points between existing posterior leads are used. This method offers the potential of tracking behavioral events in the millisecond range in comparision to the 30' of integrated cortical activity which the PET Scan displays.

MAJOR FINDINGS

Method Development

PET Scans with FDG result in a rate of glucose metabolism (GMR). Methods for appropriate, accurate, and noninvestigator biased analysis of the enormous data accumulated by this method are required. As our chief interest in behavior-brain-relationships lie in gray-matter structures the initial attempts to develop analyses were with a peel technique (described in more detail in prior annual reports) which looked at the outer gray matter mantle as determined on the basis of a specific distance from the skull.

In continuing analysis of this method in preparation for publication, and thereby peer review, several advantages to the method were reaffirmed, however, one disadvantage became evident. The peel leads to a sampling bias and therefore overestimates raw and standardized PET data. To correct for this problem, a new data extraction procedure that retains most of the advantages of the peel, but does not suffer from a sampling bias has been developed, the "ring", primarily through the efforts of John Cappelletti. Both methods insure a minimization of investigator bias in identification of anatomic regions for analysis, but lead to somewhat different estimates of group difference significance levels.

Similar concerns have led us to begin to look in detail at the issue of standardization in the analysis of PET data. Standardization is the usual route for handling raw data, when the data itself has large sources of variance that are attributable to factors that are not in of themselves considered important issues for analysis to the investigator. With Campbell Clark heading this effort, it was determined that 75 percent of total regional anatomic variability could be ascribed to individual differences in global metabolic rate (GMR), i.e., almost all of the localized GMRs were highly correlated with global GMR, whereas 15.8 percent of the total variance was attributable to regional metabolic variation, i.e., inferred regional brain activity. A standardization procedure utilizing 53 regions of interest was developed that amplifies this aspect of individual differences through use of a z score determined from the overall GMR of a subject and his own variance. The method then allows for the most appropriate comparisons of either normal individuals to this gorup pattern, another group of normals other than that for which the pattern was based on, or individual patients or patient groups to this pattern. Such an analysis, e.g., could be based on th Q-component as one alternative for decomposition of such

variability. In principle such a procedure allows for the direct quantitative determination of the similarity between any subject and a group of normals, i.e. an intersubject correlation is established. As in the instances of other multivariate analyses, finding statistically significant differences among groups or individuals would then justify a larger number of specific comparisons to establish which regions are primarily responsible for the pattern alteration.

To ascertain the best method of standardization, or if standardization is in fact useful, the analysis of normal groups undergoing different psychological tasks during the PET procedure has been undertaken. The criteria to judge the usefulness of each procedure is then based on the largest F scores that lead to differentiable group data, i.e. highest sensitivity to change in task while still maintaining a low variance between subjects doing the same task equally well. William Semple has carried these analyses to the point where we can say that it is apparent that one can see differences with absolute GMR, but that these differences, although larger than those observed using standardized GMRs, do not, in general, yield statistical significance whereas the standardized variables do. These studies have been exceptionally important for establishing the n's required for statistically reliable results. It is apparent that an n of at least 9 is necessary in dealing with homogenous groups, and therefore, with patient populations in which heterogenity is probable, a larger n is clearly required.

Efforts have just begun to develop with Nuclear Medicine several new ligands for PET use. Ideally, these ligands are designed to supply information pertaining to neurotransmitter related pathway activity. Dr. Candace Pert, working primarily with Drs. Kenner Rice, Mike Channing and Steve Larson, have demonstrated that an opiate receptor ligand acetylcyclofoxy is preferentially retained in the opiate receptor rich region of the caudate but not the opiate receptor poor cerebellum. It is also displaced stereoselectively with naloxone. Also efforts have begun to develop a dopamine receptor ligand with Dale Kiesewetter and Bill Eckelman of Nuclear Medicine. We have tested a number of new fluorinated derivatives of spiperone that retain high affinity for calf striatal dopamine receptors, and therefore are good candidates for labeling with the positron emitting isotope F18 (Kiesewetter, et al., Dopamine Receptor Ligands: Chemical Syntheses and Dopamine Receptor Affinities of Halogenated Derivatives of Spiperone, American Chemical Society, 1985).

Patient Studies With PET

The last of the data collected with somatosensory stimulation (pain) as the behavioral paradigm for the PET procedure and F18-deoxyglucose as the ligand, has begun to be analyzed, primarily with the former chief of the Section, Dr. Monte Buchsbaum, and Dr. Lynn DeLisi who has also moved from the section. The data can only be said to be weakly supportive of the hypofrontality hypothesis. Hypofrontality is observable in both the schizophrenic and affective disorder patients to about the same degree and was statistically only observable in the right hemisphere of schizophrenic patients. The statistical methods used to make these determinations were repeated measures analyses of variance, a method which may not be best suited to the anatomic distinctions of the PET methodology. In attempting to correlate specific clinical symptomatology with hypofrontality, only four of the 26 items of the BPRS, emotional withdrawal, disorientation, helplessness/hopelessness and distractability were positively correlated with hypofrontality. Family history, ventricular enlargement by CT, and total BPRS

scores did not. Negative correlations, however, would be the expected corollary of the executive function hypothesis of hypofrontality in schizophrenia as "hyperfrontality" is meant to reflect the observations in man of the higher relative GMRs in the frontal cortex in comparison to either parietal or parietal plus occipital cortex regions reflecting the prominence of the executive or planning functions of the brain of man. Only "cooperativeness", however, demonstrated a significant negative correlation. On examination of neuroleptic treated patients a "worsening" of hypofrontality was observed despite improvement in the clinical symptomatology of the patients.

Interregional correlation analyses primarily handled by Drs. Campbell Clark and Robert Kessler found significantly different relationships in schizophrenic patients and normals. Some of the most dramatic differences involved the left rolandic cortical area (the primary somatosensory cortex). In normals, significant positive correlations were observed between the GMR in this area and the GMRs of the left parietal cortical areas. In contrast, these same areas either lacked significant correlations or else demonstrated significant negative associations in the schizophrenia group. Within plane maps of correlations therefore appear consistent with the concept of a lack of "coupling" or "linkage" in response to sensory stimuli in schizophrenia.

Human Studies with Electrical Mapping

Using a 16 lead evoked potential mapping system, Dr. Henry Holcomb has been directing the study of somatosensory responses to pain stimuli in normals, schizophrenic patients and affectively disordered patients. This work has been an important complement to the PET studies which make use of a similar behavioral paradigm and allow us to compare the two forms of brain imaging within the same subject. Each subject was given a somatosensory/pain discrimination task before and after a 32 minute period of noxious stimulation of the right forearm. During that epoch evoked cortical potentials were obtained from 16 scalp electrodes. Psycho- physical pain ratings administered prior to the shock period clearly distinguish normals from patients with schizophrenia, but do not distinguish normals from patients with affective illness.

In normals, a gradual diminution of evoked electrical activity occurs in the area of the brain thought to be the associative sensory cortex. This pattern appears to represent the brain's selective habituation to a repetitive, benign predictable environmental stimulus. In contrast, electrical activity in the brains of schizophrenic patients tended to shift in location and magnitude both decreasing and increasing in an oscillating manner. The sickest patients showed an especially unusual pattern, displaying a progressive rise in activity over time. These findings may be the electrical equivalent of the differences in interregional GMR correlations reported above, i.e. the correlations between the left parietal and sensory regions and frontal regions, that are absent in schizophrenic patients but observeable in normals receiving somatosensory stimuli. (Holcomb, H.H., et al., Interregional brain metabolism in schizophrenia, New Research at American Psychiatric Association's 138 Annual Meeting, 1985, abstract number 2, page 19).

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

In the past, there have been considerable difficulties involved in trying to assess region specific functional activities as well as neurotransmitter

functional activity in the brain. PET scanning and mapping based on electrical activities provide two very exciting approaches for solving these methodologic problems. They promise to play particularly important roles in the study of psychiatric disorders where there is little evidence for structural changes in the brain. The findings so far have already begun to elucidate some aspects of the normal physiology of pain pathways and the possible psychiatrically related alterations in the same.

A concentrated effort has also been made to examine frontal cortex activation in normal and psychiatric patients as measured by the PET scan as the function of this anterior area of the cortex may relate more closely to the observed alterations of behavior in psychiatric patients. In the past, the increased blood flow observed in this region upon task initiation has been hypothesized to result from the presumed responsibility of this region for the planning of goal-directed behavior in people in comparison to posterior cortical areas which may relate more directly to sensory processes. Based on this earlier work, however, we have tried to become somewhat more sophisticated and look at overall patterns rather than just the ratios of front to back. We know, for example, that the frontal cortex has both anatomic and functional subdivisions. From our most recent work, though still in need of final collation, it is apparent that PET is able to differentiate different parts of the frontal cortex depending upon the task that the brain is organized to deal with, i.e., experimenters working in PET must appreciate what the psychological literature (behavioral response or operate conditioning literature) offers to the understanding of a particular pattern of metabolism or other maps of localization of PET isotope. In this respect, we are still far from attaining the requisite understanding of normal brain function that would enable us to feel confident in our understanding and interpretation of the abnormalities that may be present in mental illness.

It is of some interest to speculate that the unusual response habituation to pain stimulation observed in our schizophrenic subjects, might represent a more generalizable phenomenon, that might occur in other sensory modalities. Such a deficit might provide a basis for exploring one of the possible fundamental neurophysiological deficits that might be responsible for the behavioral symptoms that we have come to label schizophrenia. The capacity then to assess specific illness and task related deficits in localized brain activity provide us with an exciting and challenging tool for the elucidation of the mechanisms that underlie abnormal behavior.

PROPOSED COURSE

We need to continue to improve our methods of statistical analysis. We appear to be headed in this direction with a variety of factor and discriminant analyses which should enhance our capacity to both discover group differences among patient populations as well as allow us to examine the homogeneity of the patient populations we do scan. We need to continue our efforts to improve the hardware and software available for data analysis of imageable data at NIMH. A VAX computer has finally arrived this year and has now been installed such that the development of new graphics possibilities should be on the way. John Cappelletti has been instrumental in researching and now ordering a data base system, SIR, that should allow for the accessing of the enormous amount of imaging data base that we will be developing in the future.

In evaluating the importance of PET data, most importantly, new tracers need to be developed to extend our functional mapping capacities. Just as the neuro-

sciences have developed from anatomical concerns to neurotransmitter concerns, we as the rest of the neurosciences must try to meld the two approaches of anatomy and biochemistry to give us neurotransmitter specific anatomical information on brain function, a task for which PET is ideally suited.

We need to continue to evaluate PET findings in relation to evoked potential data, biochemical measurements, and behavioral assessments including drug response. Insofar as only small differences have been observed between psychologically disturbed patients and normals, new strategies emphasizing selective neurotransmitter challenge approaches are a logical step in the search for neurotransmitter or regional functional differences between patients and normals.

In addition, the data on somatosensory habituation should be extended to study other patient groups; e.g., phobic patients and Alzheimer patients. The electrophysiological mapping procedures facilitate the study of the psychopharmacology of habituation, a very important fundamental process of the nervous system upon which most higher cortical functions may depend.

The psychological tasks we have employed to date for PET scanning cover two fundamental neurophysiological but complementary processes, selective attention and habituation to nonrelevant stimuli. In this respect, they provide a meaningful basis to explore aspects of attention in the mental disorders, both from the perspective of what the required areas are for activation and decrement, but potentially for the chemical neuroanatomy of such function. We intend to explore this by using challenge strategies (administration of drugs believed to alter attention) and with the use of neurotransmitter specific ligands.

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Buchsbaum, M.S., Holcomb, H.H.: Positron emission transaxial tomography. In R.C.W. Hall and T.P. Beresford (Eds.): Handbook of Psychiatric Diagnostic Procedures, Spectrum Publications, Jamaica, N.Y., 1985, pp. 327-344.

Buchsbaum, M.S., Holcomb, H.H., DeLisi, L., Hazlett, E.: Brain imaging in affective disorders. In A.J. Rush and J. Altschuler (Eds.): Depression: Basic Mechanisms, Diagnosis, and Treatment, Guilford Press, New York, 1985, (in press).

Buchsbaum, M.S., Kessler, R., King, A., Johnson, J., Cappelletti, J.: Simultaneous cerebral glucography with positron emission tomography and topographic electroencephalography. In G. Pfurtschellar, E.T. Jonkman, and F.M. Lopes da Silva (Eds.): Brain Ischemia: Quantitative EEG and Imaging Techniques, Progress in Brain Research, 1984, pp. 263-269.

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Clark, C., Carson, R., Kessler, R., Margolin, R., Buchsbaum, M., DeLisi, L., King, C., Cohen, R.: Attenuate statistical models for the examination of clinical PET/FDA data. J. of Cerebral Blood Flow and Metabolism 5: 142-150, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00931-12 LGCB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characteristics and Regulation of S-adenosylhomocysteine Hydrolase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. G. L. Cantoni	Chief, Laboratory of General and Comparative Biochemistry	LGCB NIMH
Others:		
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R. R. Aksamit	Research Chemist	LGCB NIMH
C. G. Unson	Senior Staff Fellow	LGCB NIMH

COOPERATING UNITS *(if any)*

Istituto Superiore di Sanita, Rome, Italy
Istituto di Chimica Biologica, University of Rome, Italy

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Protein

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

S-adenosylhomocysteine hydrolase plays a critical role in regulating AdoMet-dependent methylations in eukaryotic cells by regulating the ratio of AdoMet/AdoHcy. Several approaches are being used to determine the structure and function of this enzyme.

1) Structure Determination: The enzyme has been purified to homogeneity from rat liver. Conformational changes for active and inactive forms of the enzyme have been examined by fluorescence and circular dichroism. Peptide fragments of the protein have been isolated and partial amino acid sequences determined. Oligonucleotide probes from the peptide sequences and antibodies are being used to screen a cDNA library from rat liver to obtain the nucleic acid sequence.
2) Ligand Binding and Kinetic Properties: The role of NAD, nucleotide, and cAMP binding in regulating the catalytic activity has been studied. A large number of adenosine and adenosylhomocysteine analogs have been examined for their ability to function as inhibitors and/or substrates of the enzyme. The two most interesting compounds studied are 3-deazaadenosine and 3-deazaaristeromycin. Both compounds inhibit the enzyme, but only 3-deazaadenosine is a substrate.
3) Inhibition of AdoHcy Hydrolase in vivo: Analogs of adenosylhomocysteine synthesized by this enzyme in vivo can form very potent and specific inhibitors of transmethylation reactions, and provide useful probes for studies on the role of specific methylation reactions in biological functions. These analogs have a wide range of biological activities, including antiviral activity against several RNA and DNA viruses, inhibition of leukocyte chemotaxis, and stimulation of cell differentiation in myoblast and erythroid cell lines.

Others Investigators (continued)

G. de la Haba	Research Scientist	LGCB NIMH
D. Carotti	Guest Researcher	LGCB NIMH
T. M. Caryk	Chemist	LGCB NIMH
S. Scarpa	Biologist, Istituto Superiore di Sanita, Rome, Italy	
R. Strom	Biochemist, Istituto di Chimica Biologica, Univ. of Rome, Italy	

Project Description:

As is well known, S-adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions. There are hundreds of reactions, each catalyzed by a specific enzyme, that utilize AdoMet as a substrate. It is obvious that the utilization of AdoMet in biological systems must be under regulatory controls, but at the present time little is known about the nature of these controls. It has been established that S-adenosylhomocysteine (AdoHcy), one of the products of transmethylation reactions that utilize AdoMet as methyl donor, is a competitive inhibitor of most reactions in which AdoMet participates. From the result of work in this and other laboratories, it has been proposed that the intracellular ratio of AdoMet/AdoHcy must be of key importance in the regulation of biological alkylation reactions, and that this ratio plays a role in determining the hierarchy of biological methylation reactions. In eukaryotes, AdoHcy is metabolized through a single metabolic pathway by S-adenosylhomocysteine hydrolase (AdoHcyase), an enzyme which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Because of the central role of AdoHcyase in the metabolism of AdoHcy and in maintaining the ratio of AdoMet/AdoHcy, this enzyme has been under intensive study in this and other laboratories.

S-Adenosylhomocysteine hydrolase has been purified from a variety of sources. Previous work has shown that the mammalian enzyme consists of structurally identical subunits, contains four mols of tightly bound NAD/mol of enzyme, and also binds cAMP and adenosine. A large number of analogs of adenosine and adenosylhomocysteine have been investigated as substrates, inhibitors, and inactivating agents of the hydrolase, and the results of these studies have provided insight into the physiological and pharmacological role of the enzyme *in vivo*. The chemistry of the catalytic reaction is fairly well understood, but very little is known about the structure of the enzyme and its relation to function. Our studies are directed towards 1) the elucidation of the primary structure of the hydrolase by molecular cloning of its c-DNA and by inference, its secondary and tertiary structure, 2) the determination of the specific polypeptide sequences that are involved in its binding, catalytic, and regulatory sites, 3) characterization of the conformational changes that accompany activation and binding modes by circular dichroism and fluorescence studies, and 4) crystallization of the enzyme by the hanging droplet technique which may provide an absolute three-dimensional structure by X-ray diffraction.

The enzyme was purified to homogeneity in milligram quantities using literature procedures, and was subjected to chemical digestion by cyanogen

bromide or enzymatic digestion with trypsin. The resulting peptide fragments were analyzed and subsequently isolated by high performance liquid chromatography. Edman degradation on a spinning cup sequencer has provided amino acid sequences to six peptide fragments to date. A mixed oligonucleotide probe based on the sequence of one tryptic fragment -Met-Met-Phe-Asp-Asn-Val-Tyr-Glu-Ala-X-Val-Gly-Val-Lys is being synthesized to screen a rat liver library. Peptide fragments from cyanogen bromide digests are in the process of being sequenced to find the amino and carboxyl termini.

The hydrolase has binding affinities for nucleosides and we are investigating the possible role of ATP, adenosine, cAMP, and the tightly bound NAD's in the regulation of the enzyme. We have shown, for example, that the enzyme is inactivated by Mg^{++}, ATP, and KCl with the loss of four molecules of NAD. However, its activity can be fully restored by incorporating only two molecules of NAD. This finding implies that only one pair of NAD's is involved in catalysis and that the other pair may be involved in regulation. It also suggests that the reactivated hydrolase may be a non-regulatory enzyme with very different properties from the native hydrolase. Fluorescence, circular dichroism, and differential ultraviolet spectroscopy studies are being done to monitor changes in conformation of the enzyme upon inactivation and reactivation. The emission and excitation spectra of inactivated enzyme, for example showed a loss in tryptophan fluorescence intensity which appears to be restored upon reactivation. ATP, adenosine, and c-AMP have binding affinities for the enzyme and it is not clear how they fit in catalysis and/or regulation. Various affinity reagents are being used to label the hydrolase which will then be subjected to specific enzyme digestion and the labeled fragments isolated and sequenced to determine the amino acid residues that comprise the active site and/or binding clefts in the protein. Cyanogen bromide and tryptic maps have already been established in our sequencing studies so that the isolation and identification of the labeled active site polypeptides will be greatly facilitated.

The purified enzyme has been used to produce antibodies against the hydrolase in rabbits. The antibodies are being used, along with the oligonucleotide probes, to screen a λgt11 cDNA library from rat liver to clone the complete nucleotide sequence for the enzyme. The cloned sequence will be used in future work to examine the expression of the gene in different cell types and during cell differentiation. The antibody against the enzyme will also be used to develop a radioimmune assay for determining levels of the enzyme in crude cell extracts.

While the biochemical mechanisms of transmethylation reactions have been elucidated many years ago, largely as a result of the studies by Cantoni and his collaborators at NIH, the correlation between many methylation reactions and cellular functions remains obscure. For instance, the significance of the methylation of a variety of informational macromolecules, such as proteins and nucleic acids (DNA, ribosomal-, messenger-, viral and tRNA, etc.), or of complex polysaccharides, or even simpler compounds such as guanido acetic acid, nicotinamide, etc., is not immediately obvious and is the subject of much debate. It can be surmised that modulation of AdoMet/AdoHcy ratio would result in important physiological effects, which if correlated with biochemical data would help reveal the significance of specific methylation reactions.

Since AdoHcyase is the only enzyme to metabolize AdoHcy in eukaryotes, inhibition of this enzyme by analogs can be used to alter the ratio of AdoMet/AdoHcy in the cell. We decided some years ago to take advantage of this fact and initiated a long range experimental project designed to study in depth the properties of AdoHcyase, and then to develop a series of specific inhibitors of this enzyme. As a result of these studies on the properties of AdoHcyase, we have established that the use of specific inhibitors makes it possible to alter the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy of the general formula S-purinylhomocysteine (PurHcy). By using these inhibitors, it is possible to modulate the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio in different cellular systems, and to examine the consequences of these changes on cellular functions.

Our studies, confirmed and extended in other laboratories, have shown that inhibitors of AdoHcyase may be divided into two groups: a) irreversible or suicidal inhibitors, and b) competitive inhibitors that inhibit the enzyme reversibly. This second group can be further classified into two subgroups; those inhibitors which can be utilized as substrates by the enzyme and those inhibitors which are not substrates. Irreversible inhibitors of AdoHcyase include the compounds 9-β-D-arabinofuranosyladenine (Ara-A), 3-deaza-9-β-D-arabinofuranosyladenine (3-deaza-Ara-A), and 2-chloroadenosine. Ara-A has been used by others in chemotherapy for cancer patients. 3-Deaza-Ara-A and 2-chloroadenosine might be expected to have clinical effects similar to Ara-A, since they produce similar inhibition of AdoHcyase. Of the many reversible inhibitors tested, two compounds have been extensively studied in this laboratory as prototype compounds of this group; 3-deazaadenosine (3-deaza-Ado) and 3-deazaaristeromycin (3-deaza-Ari). 3-Deaza-Ado is a potent competitive inhibitor of AdoHcyase with K_i of 5-8 μM, and as a substrate has a K_m value about equivalent to the natural substrate, adenosine. In contrast to 3-deaza-Ado, 3-deaza-Ari is not a substrate for AdoHcyase, but it is a very potent competitive inhibitor, with K_i of 2.0 nM for the hamster liver enzyme. Neither compound is a substrate for either adenosine kinase or adenosine deaminase.

It would be expected that the intracellular accumulation of AdoHcy and/or 3-deaza-AdoHcy, with the accompanying changes in the AdoMet/AdoHcy ratio, would result in the inhibition of a number of methyltransferases. This should cause an increase in the intracellular level of AdoMet (as a consequence of its under-utilization) and in a decrease in the intracellular concentration of many methylated intermediates. We have been able to verify this prediction, demonstrating a striking decrease in the amount of many methylated compounds, including methylated phospholipids, methylated proteins, and creatine in the liver. There is a wide range in the sensitivity of different methyltransferases to inhibition by AdoHcy and AdoHcy analogs *in vitro*. Unfortunately, cellular membranes are relatively impermeable to AdoHcy and its analogs, so it has been difficult to take advantage of the specificities of these analogs *in vivo*. However, the capacity of AdoHcyase to synthesize AdoHcy analogs *in vivo*, as has been shown with 3-deaza-Ado, demonstrates the exciting possibility of synthesizing potent and specific methylation inhibitors intracellularly.

Administration of 3-deaza-Ado to laboratory animals or to cells in culture results in the accumulation of both 3-deazaadenosylhomocysteine (3-deaza-AdoHcy) and AdoHcy. The accumulation of 3-deaza-AdoHcy can be increased by addition of

homocysteine, due to AdoHcyase acting in reverse of the normal hydrolytic direction. Since 3-deaza-Ari is a potent inhibitor of AdoHcyase, the AdoHcy accumulates in levels which reflect the rate of transmethylation reactions, and not the catalytic rate of AdoHcyase. Differences between species were observed for the metabolite levels formed after treatment with 3-deazaadenosine; in rats accumulation of both 3-deaza-AdoHcy and AdoHcy was observed, while the same treatment in hamsters resulted in an accumulation of only 3-deaza-AdoHcy. Examination of the kinetic properties of the enzyme from rat and hamster liver revealed that the K_m for AdoHcy is ten times smaller for the hamster enzyme than for the rat. This could explain the lack of AdoHcy accumulation in hamster liver.

Since 3-deazaaristeromycin has such a low K_i for AdoHcyase, high concentrations of this compound should effectively block the enzyme, preventing the conversion of AdoHcy to adenosine and homocysteine. Comparison of the effects of 3-deaza-Ado and 3-deaza-Ari on the replication of RAW264 cells showed that, at sufficiently high concentrations, 3-deaza-Ado was cytolytic after one day and that 3-deaza-Ari was cytostatic. Micromolar homocysteine reversed the cytostasis of 3-deaza-Ari, but did not reverse the cytotoxicity of 3-deaza-Ado. Since AdoHcy is the only cellular source of homocysteine, cells incubated with 3-deaza-Ari cannot recycle methyltetrahydrofolate and regenerate tetrahydrofolate for use in de novo synthesis of purines and pyrimidines. This condition is similar to the situation with vitamin B_{12} deficiency, which inactivates methionine synthase, and causes methyltetrahydrofolate to accumulate. In addition, it would be expected that cells incubated with 3-deaza-Ari would contain less cystathionine, an amino acid without a known function that is found in high concentration in the brain. These findings could have clinical significance in situations where AdoHcyase is inhibited such as the administration of Ara-A and patients with adenosine deaminase deficiency.

Comparison of the biological effects of 3-deaza-Ado and 3-deaza-Ari has made it possible to attribute some of the differences in specificity to the finding that 3-deaza-AdoHcy is a more potent and specific inhibitor of some transmethylation reactions than AdoHcy. We have found that macrophage chemotaxis is specifically inhibited by the intracellular formation of 3-deaza-AdoHcy, brought about by treatment of the cells with 3-deaza-Ado, while chemotaxis is unaffected by accumulation of AdoHcy by treatment with 3-deaza-Ari. Both 3-deaza-Ado and 3-deaza-Ari bring about a significant inhibition of methylation of phosphatidylethanolamine in these cells, which rules out a requirement for this reaction in chemotaxis. In the same manner, both compounds inhibit protein lysine and arginine methylation and carboxylmethylation to the same extent. We have further shown that inhibition of chemotaxis by 3-deaza-Ado is correlated with inhibition of the synthesis of specific proteins which are not inhibited by 3-deaza-Ari. Treatment of macrophage cells RAW264 with 3-deaza-Ado greatly inhibits rRNA and mRNA synthesis, while 3-deaza-Ari inhibits rRNA synthesis but only partially inhibits mRNA synthesis. Both 3-deaza-Ado and 3-deaza-Ari inhibit various RNA and DNA viruses, however, the sensitivity of various viruses to these two drugs is different. The specific reaction(s) involved in inhibition of RNA synthesis has not been identified, and the effect of both compounds on RNA methylation may be useful for examining the role of various methylations in the synthesis and processing of different classes of RNA.

Both 3-deaza-Ado and 3-deaza-Ari can stimulate cell differentiation in a number of cell lines, suggesting that a methylation reaction may be involved in altering gene expression in differentiation. In collaboration with Drs. Scarpa, Strom, and Bozzi, it was found that 3-deaza-Ado will increase the rate of myoblast fusion to form myotubes when the cells are placed in a permissive fusing medium. In addition, non-fusing variants of myoblast cells, which normally do not fuse in a permissive fusing medium, will fuse when 3-deaza-Ado is added. The effect of 3-deaza-Ado and 3-deaza-Ari on differentiation in a myloid cell line has also been examined. Both 3-deaza-Ado and 3-deaza-Ari effectively stimulate the synthesis of globin in these cells to an extent comparable with known inducers of globin synthesis. Work from several other laboratories has suggested that DNA methylation may be involved in expression of specific genes. It is possible that 3-deaza-Ado may cause differentiation of these cells by inhibiting DNA methylation. However, since 3-deaza-Ado also inhibits a number of other methylation reactions, further work will be required to identify the reactions involved.

In a series of recent studies in Europe and in this country, it has been found that AdoMet, given parenterally to depressed patients produced rapid and remarkable improvement in the clinical picture. These studies indicate that AdoMet has approximately the same antidepressant activity as the standard tricyclics, such as imipramine, amitryptiline, etc. It is noteworthy, however, that administration of AdoMet is not accompanied by any toxic side effects, and thus, this mode of therapy may represent a considerable improvement over the therapeutic regimens currently in use. The mechanism of action of AdoMet in depressive illness is unknown. It should be pointed out, however, that the dose of AdoMet found to be effective in the management of clinical depression (200-400 mg/i.v./day) is very small compared to the daily flow of methionine through AdoMet. Human adults synthesize and metabolize about 20 millimoles of AdoMet/day, or 20-40 times the dose used in clinical trials.

Significance to Biomedical Research and the Program of the Institute:

Studies of the AdoHcyase and its inhibitors are important to understanding the regulation and function of biochemical transmethylations, and have possible clinical applications in the development of specific inhibitors for certain methylation reactions. Since AdoMet dependent methylation reactions are involved in the synthesis of so many compounds, including DNA, RNA, proteins, lipids, and neurotransmitters, the regulation of these reactions can alter many cell functions. Inhibitors of methylation reactions have been shown to affect cell differentiation, leukocyte chemotaxis, and virus replication. The possible clinical applications could be in the development of compounds for use in chemotherapy, immunosuppression, and antiviral drugs. Because of the important role of methylation in neurotransmitter synthesis, these compounds could have important effects on brain function as well.

Proposed Course of Research:

Studies on several inhibitors will continue in order to determine specific mechanisms of inhibition, and to determine correlations between inhibition of specific reactions and the physiological effects of these compounds. Much of the work will focus on methylation reactions involved in leukocyte chemotaxis, and in RNA and protein synthesis. The differences in inhibition of lipid and protein methylation by the various compounds will continue to be examined.

Publications:

DeBlas, A., Adler, M., Shih, M., Chiang, P.K., Cantoni, G.L. and Nirenberg, M.: Novel inhibitors of CDP-choline synthesis, action potential calcium channels, and stimulus-secretion coupling. Proc. Natl. Acad. Sci. USA. 81: 4353-4357, 1984.

Backlund, P.S. Jr., Meade, B.D., Manclark, C.R., Cantoni, G.L. and Aksamit, R.R.: Pertussis toxin inhibition of chemotaxis and the ADP-ribosylation of a membrane protein in a human-mouse hybrid cell line. Proc. Natl. Acad. Sci. USA 82: 2637-2641, 1985.

Cantoni, G.L.: The role of S-adenosylhomocysteine in the biological utilization of S-adenosylmethionine. In Cantoni, G.L. and Razin, A. (eds.): Chemistry, Biochemistry, and Biology of DNA Methylation. New York, Alan R. Liss, Inc., in press.

Cantoni, G.L.: The centrality of S-adenosylhomocysteinase in the regulation of the biological utilization of S-adenosylmethionine. In The Biochemistry of S-adenosylmethionine as a Basis for Drug Design Symposium, Bergen, Norway, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00936-22 LGCB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Homocystinuria: Methionine Metabolism in Mammals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH
Others:		
F. Skovby	Visiting Associate	LGCB NIMH
H.L. Levy	Assist. Prof. of Neurology	Mass. General Hospital, Boston

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Submission and acceptance of a manuscript "The Natural History of Homocystinuria Due to Cystathionine β-Synthase Deficiency, which appeared in the American Journal Human Genetics 37:1-31, 1985, has completed this project for the time being.

No new initiatives have been undertaken on this project during the past year after completion of the homocystinuria survey.

Publications:

Skovby, F., Mudd, S.H.: Genetic diseases of sulfur metabolism in humans. In Muller, A., and Krebs, B. (Eds.): Sulfur, A Unique Element: The Significence for Chemistry, for the Geo-, Bio- and Cosmosphere and Technology. Amsterdam, The Netherlands, Elsevier Scientific Publishing Co., 1984, pp. 479-494.

Skovby, F., Mudd, S.H.: Clinical and biochemical studies of homocystinuria due to cystathionine β-synthase deficiency. In Verlag, G.T. (Ed.): Proceedings from International Symposium on Recent Progress in the Understanding, Recognition and Management of Inherited Diseases of Amino Acid Metabolism. Stuttgart, New York. (In press).

Mudd, S.H.: Homocystinuria. In Wyngaarden, J. B., and Smith, Jr., L. H. (Eds.): Cecil Textbook of Medicine. 17th ed, Philadelphia, W. B. Saunders Company, 1985, pp 1133-1134.

Mudd, S.H., Skovby, F., Levy, H.L., Pettigrew, K.D., Wilcken, B., Pyeritz, R.E., Andria, G., Boers, G.H.J., Bromberg, I.L., Cerone, R., Fowler, B., Grobe, H., Schmidt, H., Schweitzer, L.: The Natural History of Homocystinuria Due to Cystathionine β-Synthase Deficiency, American Journal Human Genetics 37:1-31, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00940-05 LGCB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methionine Biosynthesis in Higher Plants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. A. H. Datko Biologist LGDB NIMH

Other:

S. H. Mudd Chief, Section on Alkaloid Biosynthesis LGDB NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The methyl balance of growing Lemna paucicostata has been studied by experiments with radioactive methionine. Quantitatively the most important methylated end-products (and the amounts accumulated per colony, in parentheses) are: phosphatidylcholine, choline, and phosphocholine (together, 10 nmole); pectin methyl ester (3.7 nmole); chlorophyll methyl ester (1.7 nmole); neutral lipid, as yet uncharacterized, but possibly cyclopropane fatty acids (1.5 nmole). Other possible methylated end-products are quantitatively small in comparison, and, together, the above plus the 4.4 nmole/colony accumulated as protein methionine account for the total methylneogenesis of the plant. About 10% of the total S-adenosylmethionine consumption of the plant is utilized in forming S-methylmethionine. Since the latter compound is formed about 30 times faster than it accumulates, it appears to be turning over rapidly metabolically. Tracer experiments show the methyl groups of S-methylmethionine are rapidly transferred back to methionine. Phosphatidylcholine is formed by sucessive methylations of phosphoethanolamine, rather than by methylation of phosphatidylethanolamine, as is commonly held to be the case for mammals and microorganisms. If exogenous choline is available in amounts sufficient to provide for the total choline needs of the plant, transfer of methyl groups to phosphoethanolamine and its products is decreased about 90%. Overall transmethylation decreases about 50%. A series of major metabolic rearrangements is set in motion. These regulatory phenomena are now being studied.

Project Description:

During the past year, we have obtained several important results during the course of our continuing investigations of the metabolism of methionine in higher plants, using Lemna as a model.

(a) The overall methyl balance.
Work previously reported in project reports with Dr. Giovanelli as principle investigator had indicated that for each mole of methionine accumulated by the plant, about four moles of methyl group are transferred from methionine to other compounds. Thus the average methionine molecule must participate repeatedly in the cycle: methionine → AdoMet → AdoHcy → homocysteine → methionine. This conclusion was based on indirect evidence, namely, the observation that, after administration of a tracer dose of doubly labelled [^{35}S, U-^{14}C]methionine to Lemna, the ratio of ^{14}C in the methyl group to ^{35}S was decreased by the metabolic activity of the plant to approximately 20% of the original ratio. This value, together with the fact that the average colony of Lemna contains 4.4 nmole methionine, permitted calculation that 17.3 nmole of methyls must be transferred by the average colony. To confirm this conclusion, and to gain better understanding of where these methyls were being transferred, we have now carried out a series of experiments in which plants were labelled for varying periods with either [^{3}H$_3$C]-methionine (for short periods, down to 1 min) or with [^{14}CH$_3$]methionine (for longer periods, from 1 hour up to as much as 7-8 days). Methods were developed to permit analysis of the amount of the relevant radioisotope in each of the compounds to which major amounts of radioactivity were transferred after each incubation interval. The results obtained permitted calculation of the initial rate of transmethylation to each of these compounds, as well as the amounts accumulating in each. Major transmethylation end products are: phosphatidylcholine, and other methylated derivatives of ethanolamine, 10 nmole; methyl groups (tentatively characterized as pectin methyl esters by virtue of being cleaved from insoluble material by mild alkali to form volatile compounds), 3.7 nmole; chlorophyll methyl ester, 1.7 nmole; neutral lipid (as yet incompletely characterized, but possibly cyclopropane fatty acids), 1.5 nmole. Other methylated products are almost certainly formed, but the amounts are small relative to the above values. For example, methylated bases in RNA contribute 0.1-0.2 nmole. No major methyl-containing product was detected other than those listed above. The total methyl thus accounted for is approx 17 nmole/colony, in excellent agreement with the predicted value of 17.3. No indication was obtained of significant turnover of the methyls in any of the above compounds.

(b) The S-methylmethionine cycle.
In contrast, there was a rather rapid flux of methyl group into S-methylmethionine (approx 1.6 nmole/colony x doubling), but this compound did not accumulate at a commensurate rate (0.05 nmole/colony x doubling). Presumably S-methylmethionine is being formed by the action of AdoMet:methionine S-methyltransferase:

AdoMet + methionine → S-methylmethionine + AdoHcy.

The failure to accumulate suggests that S-methylmethionine is rapidly consumed metabolically. Experiments with [^{3}H$_3$C]-S-methylmethionine confirmed this expectation. Radioactivity originating in the methyl group of this sulfonium salt appeared very rapidly in methionine and thereafter spread to the expected metabolic products of methionine. To test whether S-methylmethionine was functioning as a

direct methyl donor, or its methyl group was necessarily being transferred via methionine as an intermediate, an experiment was performed in which plants were simultaneously briefly pulse-labelled with both [$^{14}CH_3$]methionine and [$^{3}H_3C$]-S-methylmethionine, then incubated for varying chase periods in nonradioactive medium. Analyses of the results of this experiment are still underway. Preliminary results indicate that AdoMet must be the methyl donor for at least major portions of the methylated ethanolamine derivatives, of pectin methyl ester, and the total methyl in neutral lipids (comprised of chlorophyll methyl esters and other neutral lipids). Overall, the results obtained to date are most compatible with S-methylmethionine utilization occurring via the following known transmethylation reaction:

S-methylmethionine + homocysteine → 2 methionine

Thus methionine appears to participate in yet a third metabolic cycle (in addition to that detailed above, and to the cycle in which it serves as the ultimate donor of the 3-carbon moiety of spermidine):

(1) Met + ATP → AdoMet + PPi + Pi
(2) AdoMet + Met → S-Methylmethionine + AdoHcy
(3) AdoHcy → Ado + Homocysteine
(4) S-Methylmethionine + Homocysteine → 2 Met

Sum: ATP → Ado + PPi + Pi

This cycle would utilize about 1.6 nmole AdoMet/colony x doubling. Since the methyl group ultimately returns to methionine, no decrease in the ratio of ^{14}C in the methyl group to ^{35}S of [^{35}S, U-^{14}C]methionine would occur as a result of the operation of this cycle, and it would therefore not have been detected in the early experiments mentioned above. Written as the sum of reactions (1-4), the cycle is a "futile" one, serving only to consume ATP. We speculate that its physiological role may be to provide a means of escaping the consequences of over-production of AdoMet. If some environmental variation alters in a major way the utilization of AdoMet (for example, as discussed below, the uptake of choline from the medium, which decreases demand for AdoMet about 50%), unless AdoMet production is very closely controlled the plant might sequester so much of the available soluble methionine pool as AdoMet that it would effectively become methionine depleted. Operation of the S-methylmethionine cycle nicely returns the relevant portions of AdoMet to methionine and prevents such depletion.

(c) A new pathway for phosphatidylcholine formation.
During the experiments in which plants were labelled with [$^{3}H_3C$]methionine for short periods, an unexpected finding was made. At early times major portions of radioactivity appeared in negatively charged compounds travelling during electrophoresis near phosphocholine. Only later did the radioactivity make its way into phosphatidylcholine. Several lines of evidence have now been adduced which indicate that the methylation steps involved in phosphatidylcholine synthesis occur at the level of phosphoethanolamine and its derivatives, rather than at the level of phosphatidylethanolamine, as is commonly held to be the case for mammals and microorganisms: (i) The above described early labelled negatively charged compounds have now been demonstrated to consist of a mixture of phosphomethylethanolamine (phospho-MEA), phosphodimethylethanolamine (phospho-DMEA), and phosphocholine. At early times the specific radioactivity of these compounds is much higher than that of phosphatidylcholine. (ii) With longer labeling times,

phosphatidylcholine becomes the dominant methylated product. However, phosphatidylmethylethanolamine and phosphatidyldimethylethanolamine could not be detected at any time. (iii) A pulse chase experiment with [^{14}C]ethanolamine showed that this compound is rapidly phosphorylated, converted to a mixture of phospho-MEA, phospho-DMEA, and phosphocholine, and to phosphatidylethanolamine and phosphatidylcholine. Again, phosphatidyl derivations of MEA or DMEA were not detected. (iv) Soluble, nonphosphorylated derivatives of ethanolamine contained very minimal amounts of radioactivity at all times. Small amounts of radioactivity appeared in soluble choline with prolonged labelling times. Together, these results indicate that the transmethylations which account for the major proportion of all choline formed in nature (since de novo formation of choline derivatives is a relatively minor pathway in mamals) occurs with soluble phospho-base as methyl acceptor.

(d) Regulation by choline.
Realization of the quantitatively dominant role of phosphatidylcholine as a methylation end-product, together with our demonstration (summarized in last year's annual report) that Lemna possesses a very active and specific system for the uptake of choline from the medium, led one to predict that choline might regulate the rate of its own biosynthesis. This prediction has now been confirmed. Lemna plants were pregrown for several generations in the presence of 25 μM choline. At this concentration the plants appear normal and have a doubling time within control limits. They take up about 20 nmole choline/colony x doubling, several times the approx 3 nmole total choline contained by control plants grown on unsupplemented medium. When such plants were labelled for various lengths of time with [$^{3}H_3$C]methionine or [$^{14}CH_3$]methionine it was found that incorporation of radioactivity into methylated phosphoethanolamine derivatives was very much decreased to about 7-9% of that found in control plants, whereas methyl transfer to form pectin methyl ester, chlorophyll methyl ester, and other neutral lipids was virtually unchanged. Thus external choline can reduce methyl flux into choline by about 90%, and reduce the overall requirement for methyl groups from 17 nmole to approx 8 nmole/colony x doubling. The results of experiments in which plants were labelled to isotopic equilibrum with either [^{14}C]choline or ^{32}Pi while growing in 25 μM choline indicate the following contents of choline derivatives (in nmole/colony, with control values in parentheses): phosphocholine, 2.0 (0.16); soluble choline, 16.4 (0.27); phosphatidylcholine, 2.7 (2.6). Thus, phosphocholine concentration has increased 12-13 fold, soluble choline 61-fold, and phosphatidylcholine virtually not at all. Similar experiments with plants labelled to isotopic equilibrum with $^{35}SO_4^{2-}$ failed to show significant differences between control and 25 μM choline-grown plants with respect to the tissue concentration of AdoMet or S-methylmethionine. Thus exogenous choline has brought about a profound change in methionine metabolism, decreasing the demand for methyl groups by 9 nmole/colony x doubling. AdoMet metabolism has apparently been regulated so that the decreased demand is not balanced by an accumulation of unused AdoMet (which would result in the death of the plant due to methionine depletion), and so that other transmethylation reactions continued essentially unperturbed. The existence of a variety of important regulatory features is thus suggested which require further experimental exploration.

Significance of Biomedical Research and to the Program of the Institute:

This project is part of our general program to investigate the aspartate biosyn-

thetic pathway in higher plants. The general significance of this research has been set forth in the report on the "Pathways of methionine and threonine metabolism, and their control, in higher plants," by Dr. Giovanelli.

Proposed Course of Research:

The results obtained during the past year raise a variety of questions which require further experimental exploration:

(a) The proposed AdoMet:phosphoethanolamine methyltransferase. We are hypothesing the existence of a hitherto undescribed methyltransferase (or series of methyltransferases) responsible for converting phosphoethanolamine to phosphocholine. These reactions may be among the quantitatively most important transmethylations in all of nature. Direct demonstration of the relevant enzyme activities and exploration of their properties thus becomes a matter of high priority.

(b) The enzymes of the S-methylmethionine cycle. Our findings lead us to postulate the existence in Lemna of two enzymes: AdoMet:methionine S-methyltransferase and S-methylmethionine:homocysteine S-methyltransferase. Direct demonstration of these activities would be important to provide additional evidence for the operation of the proposed S-methylmethionine metabolic cycle.

(c) The transmethylases forming pectin methyl ester, chlorophyll methyl ester. Demonstration of the methyl donor specificities of these enzymes now becomes important to further evaluate whether S-methylmethionine can function as a direct methyl donor, or AdoMet solely fulfills this function.

(d) S-Adenosylmethionine synthetase. Reasons for investigation of this enzyme in Lemna were presented last year. An important additional tool is now at hand which seems likely to lead to interesting and significant regulation of the enzyme: choline, which reduces the overall demand for AdoMet by about 50%, leaves transmethylations other than those leading to phosphatidylcholine unaffected, and in the presence of which AdoMet concentration is virtually unchanged. The need for exact regulation of the AdoMet concentration is emphasized by the realization that unless this occurs the plant is extremely susceptible to functional methionine depletion.

(e) The earlier steps in phosphoethanolamine synthesis. How is this compound (the proposed intermediate leading to phosphatidylcholine) formed? Decarboxylation of serine, followed by phosphorylation is possible. An alternative pathway would involve decarboxylation of phosphoserine. Definition of this sequence is important because one primary site at which choline regulates phosphatidylcholine formation is likely to lie in this early sequence.

(f) Choline appears to down-regulate not only its own biosynthesis but overall methyneogenesis. At which site or sites is this regulation exerted? What are the effectors? Understanding of these areas would provide deeper insight into the control of methylneogenesis. Choline regulation has the additional favorable feature that, rather than being solely a laboratory artifact, it appears to be a phenomenon which occurs in Lemna growing in nature under normal physiological conditions.

Publications:

Datko, A.H., Mudd, S.H.: Uptake of amino acids and other organic compounds by *Lemna paucicostata* Hegelm. 6746. *Plant Physiology* 77:770-778, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00942-04 LGCB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Reactions in Mammalian Cell Chemotaxis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	G. L. Cantoni	Chief, Laboratory of General and Comparative Biochemistry	LGCB NIMH

Others:

R. R. Aksamit	Research Chemist	LGCB NIMH
P. S. Backlund, Jr.	Senior Staff Fellow	LGCB NIMH

COOPERATING UNITS *(if any)*

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Division of Bacterial Products, CDB, FDA

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chemotaxis by the RAW264 mouse macrophage cell line was inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin. A search for biochemical reactions inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin has revealed that only one reaction, the synthesis of a small number of proteins identified after separation by two-dimensional polyacrylamide gel electrophoresis, has the necessary inhibitor specificity for involvement in the 3-deazaadenosine-sensitive step of chemotaxis. A study with several adenosine analogs showed a correlation between inhibition of chemotaxis and inhibition of the synthesis of a common subset of proteins. These analogs also inhibited the synthesis of polyadenylated mRNA, leading us to postulate that incubation of cells with 3-deazaadenosine inhibits a methylation reaction that is required for the formation of a functional mRNA coding for one or more proteins required for chemotaxis.

Experiments to identify attractant-specific proteins have been limited because chemically defined attractants for RAW264 cells have not been available. This problem has been overcome by the isolation of a stable cell hybrid from a fusion between human leukocytes and a thioguanineresistant RAW264 cell line. The hybrid expressed functional genes for chemotaxis to N-formylmet-leu-phe, a commercially available synthetic attractant. The hybrid has been used to show that chemotaxis is inhibited by pertussis toxin. For inhibition of chemotaxis, pertussis toxin must enter the cell and ADP-ribosylate a 41,000 molecular weight protein.

Studies of the inhibition of chemotaxis by pertussis toxin and cholera toxin have led us to conclude that guanine nucleotide binding proteins are involved in transduction of the chemotactic signal. Guanine nucleotide binding proteins do not couple chemoattractant receptors to adenylate cyclase. This conclusion is based on the finding that there is no correlation between inhibition of chemotaxis and levels of cAMP and on the observation that attractants do not change cAMP levels or alter the activity of adenylate cyclase.

Other Investigators:

D. Carotti	Guest Researcher	LGCB NIMH
T. M. Caryk	Chemist	LGCB NIMH
L. Harvath	Research Microbiologist	OB NCI
B. D. Meade	Research Microbiologist	DBP FDA

Project Description:

The important discovery in this laboratory that chemotaxis by a macrophage cell line is specifically inhibited by 3-deaza-AdoHcy has allowed us to assess the significance of certain biochemical reactions in macrophage chemotaxis. Our conclusion was based on the finding that RAW264 chemotaxis is inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin, and a search was initiated for a biochemical reaction that also showed this inhibitor specificity.

The synthesis of phosphatidylcholine by methylation of phosphatidylethanolamine, the release of arachidonic acid when cells are incubated with EAMS (endotoxin-activated mouse serum, an attractant for mouse macrophages), methylation of the lysine and arginine residues of protein, and protein carboxymethylation were all inhibited by both 3-deazaadenosine and 3-deazaaristeromycin. From these studies we conclude that none of these reactions are required for chemotaxis by RAW264 cells.

In contrast, the synthesis of one or a small number of proteins, identified after separation by two-dimensional polyacrylamide gel electrophoresis, does show the necessary inhibitor specificity for involvement in RAW264 chemotaxis. Quantitation of 100 of the more prominent proteins on the gels by computerized densitometry showed that in cells treated with 3-deazaadenosine the synthesis of approximately 10% of the proteins was inhibited by more than 50%, whereas in cells treated with 3-deazaaristeromycin the synthesis of these proteins was not significantly inhibited. The correlation of the inhibition of a subset of proteins with the inhibition of chemotaxis was strengthened by the finding that other inhibitors of chemotaxis inhibited the synthesis of the same subset of proteins. These inhibitors are 3'-deoxyadenosine and the combination of erythro-9-(2-hydroxy-3-nonyl)-adenosine (EHNA), adenosine and homocysteine. A common feature of the inhibitors of chemotaxis described above is that they all can inhibit the synthesis of functional mRNA. In this regard, we have also found that inhibitors of protein synthesis and translation, such as cycloheximide, puromycin and actinomycin D, inhibit chemotaxis.

We have proposed as a working hypothesis that treatment of RAW264 cells with 3-deazaadenosine, 3'-deoxyadenosine, and the combination of EHNA, adenosine and homocysteine inhibit the synthesis of functional mRNA coding for one or more chemotactic proteins. In support of this hypothesis, we have found that 3-deazaadenosine is a more potent inhibitor of polyadenylated mRNA than 3-deazaaristeromycin and that AdoHcy and 3-deazaAdoHcy do not inhibit *in vitro* translation.

Time-lapse video cinematography shows that motility and EAMS-induced morphological changes are similar in 3-deazaadenosine-treated and control cells. These observations suggest that in cells treated with 3-deazaadenosine, signal processing after attractant binding to the chemoreceptor is inhibited.

Additional studies to examine directly the effects of 3-deazaadenosine on attractant binding or to investigate the steps in signal transduction have been hindered by the lack of chemically defined attractants. The attractants described for RAW264 cells, EAMS and LDCF (lymphocyte-derived chemotactic factor), are both complex molecular mixtures with multiple biological activities. On the other hand, human monocytes and neutrophils are known to exhibit chemotaxis to FMLP (N-formylmet-leu-phe), a commercially available synthetic attractant. For these reasons hybrids cells were isolated from fusions between human leukocytes and thioguanine-resistant RAW264 cells, and many of the hybrids exhibited chemotaxis to FMLP. Initial characterization of one of these hybrids (WBC264-9) shows that chemotaxis to both FMLP and EAMS is inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin, indicating that data obtained with these inhibitors for RAW264 may also be applicable to the hybrid cells. The WBC264-9 cell line has been cultured for more than 6 months without loss of chemotaxis to FMLP demonstrating that a stable cell line has been obtained. This cell line should allow us to identify and characterize attractant-specific reactions.

A study relating to the human FMLP receptor has been carried out in collaboration with Dr. L. Harvath on the effects of oxidized FMLP on chemotaxis and the generation of superoxide anion by human neutrophils and monocytes. This laboratory's principal contribution has been the preparation and analytical determination of chemical derivatives of FMLP. These studies have shown that human monocytes exhibit chemotaxis for both FMLP sulfoxide and sulfone, whereas human neutrophils do not exhibit chemotaxis to either of the oxidized peptides. In contrast, both human neutrophils and monocytes migrate to FMLP and both cell types generate superoxide anion and release enzymes when stimulated with FMLP, FMLP sulfoxide or FMLP sulfone. These data suggest that the FMLP receptor complex or chemotaxis transduction mechanism is different in human neutrophils and monocytes.

Reports in the literature and results from this laboratory suggest that an interrelationship between cAMP and AdoHcy metabolism may exist. During the course of studies designed to examine the effects of increased levels of cAMP upon the activity of AdoHcy hydrolase, it was found that chemotaxis by RAW264 cells was not inhibited when intracellular cAMP was increased by teatment of the cells with forskolin or isoproterenol. However, the chemotaxis of cells treated with cholera toxin, which raised intracellular cAMP to levels that were comparable to those achieved by forskolin and isoproterenol, was inhibited. These data show that increased levels of cAMP per se do not inhibit chemotaxis and suggest that either the binding of cholera toxin to the cell surface or the ADP-ribosylation reaction catalyzed by cholera toxin may be involved in the inhibition of chemotaxis. In this regard, RAW264 chemotaxis is also inhibited by pertussis toxin, another bacterial protein with ADP-ribosylating activity.

Significance of Biological Research to the Program of the Institute:

Several reports have shown that stress-induced neuropeptides modulate immunological activities and that leukocytes have receptors for beta-endorphin. Chemotaxis is an important component of the immunological response, and it has been shown that human monocytes exhibit chemotaxis to met-enkephalin and beta-endorphin. Injection of beta-endorphin into the rat cerebral ventricle results in the immigration of macrophage-like cells, indicating that chemotaxis to beta-endorphin

can occur in vivo. Identification of the steps involved in chemotaxis would provide a basis for the development of strategies to counteract stress-induced immunological dysfunction.

Mammalian cell chemotaxis is also important in the development of the nervous system, inflammation and wound healing, and chemotaxis is a behavioral response at the cellular level. Studies of bacterial chemotaxis from the laboratories of Koshland and Adler have shown that bacteria have "memory" and adapt to their environment, and progress has been made in explaining these concepts in molecular terms. The mammalian cell line model for chemotaxis that we have developed provides a mammalian system to test concepts developed from bacterial chemotaxis and to study the biochemical reactions involved in signal transmission.

Proposed Course of Research:

Future work will be directed toward verification of the hypothesis that 3-deaza-AdoHcy specifically inhibits the synthesis of functional mRNA coding for one or more chemotactic proteins, and toward the indentification of biochemical reactions important in chemotaxis. These problems will be approached by a combination of biochemical and genetic techniques.

Publications:

Harvath, L. and Aksamit, R.R.: Oxidized N-formylmethionyl-leucyl-phenylalanine: Effect on the activation of human monocyte and neutrophil chemotaxis and superoxide production. J. Immunol. 133: 1471-1476, 1984.

Aksamit, R.R., Backlund, P.S. Jr. and Cantoni, G.L.: Cholera toxin inhibits chemotaxis by a cAMP independent mechanism. Proc. Natl. Acad. Sci. USA, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00943-04 LGCB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathways of Methionine and Threonine Metabolism and Their Control in Higher Plants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. J. Giovanelli	Research Chemist	LGCB NIMH
Others:		
S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH
A. H. Datko	Biologist	LGCB NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

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Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Preparation for publication of a large backlog of research findings has been completed.

The physiological effects of the potent inhibitions of threonine synthase by P_i and AMP, and of cystathionine γ-synthase by P_i, were examined. Each of the inhibitions was competitive with O-phosphohomoserine. Tissue P_i and AMP were determined, and concentrations estimated for chloroplasts, the organelle in which threonine synthase and cystathionine γ-synthase are localized. Calculations indicated that for growth at standard external P_i or above, if the substrates (O-phosphohomoserine, cysteine) and the effector S-adenosylmethionine were uniformly distributed within plants, total activities of the two enzymes in question would fall two orders of magnitude below the amounts required to provide threonine and methionine adequate for growth. One factor, namely an increase in the concentration of O-phosphohomoserine, has the potential to restore the activities of both enzymes to meet physiological needs. If O-phosphohomoserine were restricted to chloroplasts, these inhibitions would be much less severe, resulting in activities approaching the required physiological amounts.

Exploratory studies were initiated to elucidate the in vivo regulatory patterns of threonine biosynthesis. Threonine synthesis appears to be at least partially regulated. Regulation of isoleucine synthesis was demonstrated at a site or sites distal to threonine. Plants supplemented with methionine contained normal concentrations of soluble threonine, arguing against the proposal that an increased concentration of soluble threonine is a key step in regulation of methionine biosynthesis, and further suggesting that methionine may regulate synthesis of O-phosphohoserine in addition to its established control at cystathionine γ-synthase.

Project Description:

(a) Preparation for publication of a large backlog of research findings has been completed, resulting in three publications, one manuscript in press, and one manuscript about to be submitted.

(b) Threonine synthase and cystathionine γ-synthase are of special interest in our studies on the regulation of methionine and threonine biosynthesis, since they catalyze the first committing steps in the synthesis of these amino acids. We have previously reported that P_i is a potent inhibitor in vitro of threonine synthase, and to a lesser extent of cystathionine γ-synthase, and that threonine synthase is also strongly inhibited in vitro by AMP. The physiological effects of these inhibitions were examined with the aim of : (i) better understanding the conditions under which these enzymes operate in vivo, and (ii) determining whether the inhibitions might be physiologically important in balancing fluxes into the methionine and threonine branches. Inhibition of threonine synthase and cystathionine γ-synthase by P_i, and of threonine synthase by AMP, was each competitive with O-phosphohomoserine. For Lemna grown at several P_i concentrations, tissue P_i and AMP were determined, and concentrations estimated for chloroplasts, the organelle in which threonine synthase and cystathionine γ-synthase are localized. Calculations indicated that for growth at standard external P_i (0.4 mM) or above, if the substrates (O-phosphohomoserine, cysteine) and the effector S-adenosylmethionine (AdoMet) were uniformly distributed within plants, total activities of the two enzymes in question would be severely inhibited, and would fall two orders of magnitude below the amounts required to provide threonine (plus isoleucine) and methionine adequate for growth. One factor, namely an increase in the concentration of O-phosphohomoserine, does have the potential to restore the activities of both enzymes to meet physiological needs. If O-phosphohomoserine were restricted to chloroplasts, these inhibitions would be much less severe, resulting in activities approaching the required physiological amounts. Even up to 50 mM external P_i, this ion does not limit synthesis of threonine or methionine in vivo sufficiently to retard growth. At concentrations of P_i above standard, O-phosphohomoserine may accumulate to higher than normal concentrations in chloroplasts, thereby contributing to maintenance of fluxes into threonine and methionine.

(c) To date, efforts to determine whether threonine feedback regulates its own synthesis have focused on threonine synthase, since this enzyme is considered a priori to be a likely regulatory site in that it catalyzes the first committing step in the threonine biosynthetic branch. Somewhat surprisingly, these studies failed to establish any regulatory property of threonine synthase that would allow threonine to regulate specifically its own synthesis. Studies were therefore initiated to establish whether synthesis of threonine (and isoleucine) is in fact regulated in vivo, and if so, to localize the regulatory sites. An initial series of experiments have been completed in which amounts of threonine (and its product, isoleucine) synthesized by the plant were calculated by the amounts of dilution in specific activities of [^{14}C]threonine fed exogenously. These exploratory experiments indicate that: (a) Growth of Lemna on supplementary threonine reduced threonine synthesis approximately one-half, consistent with feedback regulation by threonine. (b) Growth on supplementary isoleucine (or isoleucine plus threonine) reduced conversion of threonine to isoleucine to

20% or less that of control plants. Within the accuracy of the determinations, supplementary isoleucine reduced the synthesis also of threonine. These findings clearly establish that isoleucine feedback regulated its own synthesis at a site or sites distal to threonine, and suggest that the decreased flux between threonine and isoleucine is accompanied by a corresponding decrease in synthesis of threonine. (c) Plants supplemented with methionine contained normal concentrations of soluble threonine. This finding argues against the commonly accepted hypothesis that an increased concentration of soluble threonine (resulting from allosteric stimulation of threonine synthase by AdoMet) is a key step in regulation of methionine biosynthesis in plants. The finding further suggests that methionine may regulate synthesis of O-phosphohomoserine in addition to its established control at cystathionine γ-synthase.

Significance to Biomedical Research and the Program of the Institute:

The primary goal of this project is to elucidate the pathways for methionine and threonine metabolism, and their control, in higher plants, using *Lemna* as an experimental system. Methionine and threonine biosynthesis are closely related in plants, the two pathways branching at the common intermediate O-phosphohomoserine. There are now a number of indications that regulation of the two biosynthetic branches may also be interrelated. Our research on each of these two amino acids therefore continues to be directed along parallel and complementary lines. This project is significant to the research goals of the Institute since methionine and threonine are among the four most commonly limiting essential amino acids in the human diet. Deficiency of these amino acids (especially during early life) in protein-calorie malnutrition may be accompanied by irreversible retardation in mental development. Plant proteins provide the source of these two amino acids almost entirely, either directly by ingestion of plant material, or indirectly through an animal intermediate. Many of the plant foods most used by man are deficient in one or both of the amino acids, methionine and threonine. An understanding of the patterns of control of the biosynthesis and metabolism of methionine and threonine will provide a rational basis for maximizing the production of these essential dietary components.

Proposed Course of Research:

(1) The preliminary suggestion that threonine feedback regulates its own synthesis *in vivo* will be quantitated more precisely. The regulatory mechanism and loci will be elucidated by determining the labeling patterns from [^{14}C]-aspartate (an early intermediate) and [^{14}C]homoserine (a late intermediate immediately preceding the branch at O-phosphohomoserine). Two main schemes for regulation of threonine biosynthesis may be proposed. In the first, regulation is distal to homoserine, and predicts identical labeling patterns from [^{14}C]aspartate and [^{14}C]homoserine in control and threonine-supplemented cultures, with either labeling pattern providing a valid measure of the relative fluxes into the threonine and methionine branches. In the second, regulation occurs at the step catalyzed by threonine-sensitive aspartokinase, with channelling of products directly into the threonine branch. This scheme predicts dissimilar labeling patterns from [^{14}C[aspartate and [^{14}C[homoserine, with only the former giving a measure of relative fluxes into the two branches.

(2) The findings described above indicate that the concentrations of P_i and AMP in chloroplasts (the site of threonine synthase and cystathionine γ-synthase) require that O-phosphohomoserine be confined to this organelle in order for sufficient amounts of threonine and methionine to be synthesized for growth. Because of the potential importance of O-phosphohomoserine, P_i and AMP in determining fluxes into the threonine and methionine branches, concentrations of these compounds will be determined directly in chloroplasts isolated from Lemna growing under various concentrations of P_i. Chloroplasts will be isolated by a non-aqueous technique that permits retention of small molecules within chloroplasts.

(3) We hope to return to a number of interesting problems raised during earlier studies on cystathionine γ-synthase. Unequivocal identification of the location of cystathionine γ-synthase on one- or two-dimensional gels should be feasible by comparing the electrophoretic patterns of partially purified enzyme from up-regulated versus down-regulated cultures. Location of the protein corresponding to cystathionine γ-synthase should make it possible to examine the molecular mechanisms (e.g. repression, covelent modification, etc.) by which methionine regulates cystathionine γ-synthase activity. The availability of even small amounts of pure cystathionine γ-synthase would further permit production of antibody to the enzyme. This antibody would open up a variety of studies such as determination of the rates of synthesis and turnover of the enzyme, and isolation of mRNA for cystathionine γ-synthase from nascent ribosomes, which could be used for synthesis of a cDNA probe for the enzyme. A major question yet to be answered is the chemical nature of the effector for down-regulation of cystathionine γ-synthase. Likely effectors include methionine and its products, AdoMet and S-methylmethionine sulfonium. Inhibitors are available that have the potential of allowing the relative amounts of these compounds to be varied. Correlation of the relative concentrations of each compound with the extent of down-regulation of cystathionine γ-synthase should permit the relative importance of each of these compounds to be assessed.

Publications:

Giovanelli, J., Veluthambi, K., Thompson, G.A., Mudd, S.H., and Datko, A.H.: Threonine synthase of Lemna paucicostata Hegelm. 6746. Plant Physiol. 76:285-292, 1984.

Giovanelli, J., and Mudd, S.H.: Radioactive methionine: determination, and distribution of radioactivity in the sulfur, methyl and 4-carbon moieties. J. Biochem. Biophys. Methods 11:1-11, 1985.

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Giovanelli, J., Mudd, S.H., and Datko, A.H.: Quantitative analysis of pathways of methionine metabolism and their regulation in Lemna. Plant Physiol. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01035-17 LMB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Process of Lysogeny

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. A. Nash	Medical Officer	LMB, NIMH
Others:	P. Kitts	Visiting Fellow	LMB, NIMH
	E. Richet	Visiting Fellow	LMB, NIMH
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	G. Zon	Dir., Molecular Pharma. Br.	MPB, FDA
	H. Weissbach	Director of Research	Roche Institute
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	R. Gumport	Prof. Dept. of Chemistry	Univ. of Illinois

COOPERATING UNITS *(if any)* Division of Biochemistry & Biophysics, Center for Drugs & Biologics, FDA; Roche Institute of Molecular Biology, Nutley, NJ; Lineberger Center Research Center, University of North Carolina, Chapel Hill, NC; Departments of Microbiology & Biochemistry, University of Illinois, Urbana, IL

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied several features of the mechanism of site-specific genetic recombination, focusing on the reaction that inserts the DNA of bacteriophage lambda into the chromosome of its E. coli host. The role of supercoiling has been explored by probing for the melting of DNA around the site of recombination crossover. The sequence features that determine the interaction of a recombination protein, IHF, with its target have been studied by chemically synthesizing variants of the normal site and biosynthetically incorporating them into substrates for recombination. These oligonucleotide mutagenesis studies confirm the features deduced from comparison of naturally occurring DNA binding sites for IHF and indicate some unexpected flexibility in the protein-DNA interaction. The relationship between the recombination that integrates lambda DNA and that which reverses the integration process (excision) has been explored. Artificial substrates show that both recombinations show the same demand for homology in a short region around the crossover site; in this region at least one strand from each parent must be identical. The result implies that both recombinations, although they have different protein requirements, use similar mechanisms.

Objectives:

Recombination between specific DNA sequences is a widespread and important source of genetic variation. In many organisms such site-specific recombination is used to change the position of genes so as to turn their expression on and off. Recombination is also used in nature to accomplish the construction of complex genes from component pieces, most notably in the generation of antibodies. This kind of genetic rearrangement is also used to join two otherwise independent DNA elements (episomes). The latter reaction is typified by the integration of bacteriophage lambda. The genome of this virus exists inside the cell as a circular DNA; to insure replication and partition of the viral DNA in synchrony with its bacterial host, the virus has evolved a site-specific recombination system that inserts the viral chromosome into the host DNA. This laboratory has been studying the biochemical nature of the insertion process. We have identified and purified the proteins that take part in this reaction and have demonstrated their interactions with the target DNA. This work has conclusively showed that genetic exchange occurs by physical transfer of DNA strands between the recombining partners. Such transfer is accomplished by Int, a viral protein that can reversible break and reseal DNA. Our current efforts are focused on how each DNA partner is activated to initiate recombination and how the two DNAs are brought together so that the breakage of DNA by Int leads to exchange of genetic information.

Major Findings:

An early result of our biochemical studies was that the substrate DNA for lambda integrative recombination must be supercoiled to be maximally effective; subsequent studies have shown that such supercoiling is essential for many processes in E. coli. Although much is now known about the enzymology of supertwisting, the reason that it is required for so many reactions remains obscure. We have now investigated the possibility that supercoiling is used in recombination reactions to assist the melting of a critical stretch of DNA. Such a possibility is supported by theoretical considerations of the strain introduced into DNA by supertwisting and its relief upon denaturation of DNA. To test this hypothesis we reacted DNA with bromoacetaldehyde, a reagent that covalently attacks single-stranded but not double-stranded DNA. We find that supercoiling does indeed lead to a bromoacetaldehyde-sensitive structure in viral DNA. A variety of mapping techniques places the sensitive region about 50 base pairs from the crossover point for integration, within the region of DNA required for function of the viral recombining site (attP). Bromoacetaldehyde modification is observed only with the supercoiled attP and not with non-supercoiled DNA containing attP nor with supercoiled DNA containing attB, the host recombining site. This latter observation is in agreement with the earlier finding that integrative recombination requires supercoiling only of attP. Although our discovery seemed promising as an explanation for the role of supercoiling in recombination, a detailed investigation has shown a poor correlation between the melting of DNA induced in attP by supercoiling and activation of attP for recombination. Specifically, bromoacetaldehyde modification is observed only at temperatures above 37° C while recombination is best observed at temperatures below 31° C; the modification is blocked by concentrations of polyamines that stimulate recombination and is blocked by IHF, a host protein that is required

for recombination. Additional studies have failed to reveal any new sites of melting in response to the binding of recombination proteins to attP. We believe our findings argue against the hypothesis that supercoiling is required to melt segments of recombining DNA and we must look elsewhere for an important consequence of this mechanical strain.

The viral attachment site (attP) displays a bewilderingly complicated array of binding sites for recombination proteins. Int binds to four regions of attP and IHF, a bacterial protein required for integration, binds to three regions that are interdigitated with the Int binding sites. A unique DNA sequence is repeated at each of the three sites of interaction with IHF. IHF also binds to this sequence (or close relatives of it) in non-attachment site DNA. A compendium of all IHF binding sites suggest that the sequence YNYAANNNNTTGQTQ (where Y stands for a pyrimidine, Q for an A or T, and N for any nucleotide) is necessary and sufficient to bind IHF. We are using oligonucleotide-directed mutagenesis to test this hypothesis. In this method, we employ a chemically synthesized stretch of DNA that has one or two base changes from the sequence found at an IHF binding site. This oligonucleotide is used as a primer for DNA synthesis on a single-stranded template containing the wild-type site. E. coli is then transfected with the resulting duplex DNA and the desired mutant is cloned. In this way we have introduced specific base changes into one IHF binding site. We then assay the mutant sites for function in two ways - DNAase footprinting to determine the capacity of the site to bind IHF and in vitro recombination to test the effect of the mutant on the function of the site. Our results show that the mutations fall into two classes: class I are those changes that permit strong binding of IHF and have little effect on recombination while class II are those that depress binding of IHF dramatically and display very decreased recombination. Most base changes have an effect that is consistent with their alteration of the consensus sequence but at least one (a T to C change at position 10) shows that the binding site for IHF has some flexibility beyond that predicted by the consensus sequence. We have applied the information gained from the study of the mutagenized site to design changes in the two other IHF binding sites of attP. Preliminary studies show that inactivation of these sites also depress recombination. Thus, we have shown for the first time that (in contrast to Int where there is redundancy in the binding sites) all three IHF sites are needed for maximal recombination.

An important aspect of site-specific recombination concerns the reversibility of the reaction. To achieve efficient insertion of a viral genome, integration of lambda DNA must not be rapidly followed by excision of the integrated DNA. It has been known for many years that lambda integration is irreversible; excision requires the participation of an additional viral protein, Xis, that is not needed for integration and is accordingly not made early after infection. The biochemical basis of this irreversibility, i.e., the role for xis protein is not obvious. The attachment sites of the integrated virus (or prophage) contain all the sequences needed to bind Int and IHF that the unintegrated virus (attP) contains, albeit in a permuted form. We do not know if the permutation of these binding sites are inconsistent with the integration pathway and demands a substantially different mechanism or whether Xis simply permits the integration pathway to be used by the permuted sites. Recently, workers at the University of Illinois have discovered a surprising feature of lambda integrative recombination. It had been shown earlier that efficient integration

requires attB and attP to be homologous for a stretch of seven base pairs around the crossover site. The University of Illinois team found that recombination works quite efficiently even if attB is not a perfectly Watson-Crick paired duplex but contains one to three mismatched bases. As long as one strand of attB DNA matchs perfectly with one strand of attP DNA, recombination proceeds efficiently. Although a detailed interpretation of this result in terms of recombination geometry is still forthcoming, it provides a characterization of integrative recombination that can be compared and contrasted with Xis-promoted excisive recombination. Accordingly, we have constructed suitable clones of the prophage site attL and generated perfectly matched and mismatched duplex attachment sites. These artificial constructs were used as recombining partners in in vitro reactions with a conventional source of the other prophage site (attR). The experiments were designed so that the attR duplex matched the base sequence of the attL DNA in 0, 1, or 2 strands. Our results are identical with those found for integrative recombination - if at least one strand of attR matches a strand of attL, recombination is maximally efficient. If neither strand of attR is identical to a strand of attL, recombination is inefficient. The result implies that the search for homology between recombining partners, a critical step in the recombination reaction, uses a similar mechanism during integration and excision. This suggests that the fundamental pathway of recombination is the same in the two directions and that irreversibility is expressed simply at the initiation of recombination.

Significance to Biomedical Research and the Program of the Institute:

Each of the studies outlined above involves an area of molecular biology where there is agreement that the phenomena are widespread and important but little is known about mechanism.

Supercoiling is universal in prokaryotes and a growing body of literature suggests that it is used to selectively activate eukaryotic genes. As yet, there is no convincing demonstration of a biochemical rationale behind the biological role of supertwisted DNA. Our demonstration that the melting of DNA, although present, is uncorrelated with potential for recombination eliminates one widely held view and clears the way for consideration of other hypotheses.

The specific interaction of proteins and DNA is subject of wide interest. Our studies using oligonucleotide mutagenesis is one of the first to utilize this technique to analyze a binding site and is providing valuable information about the points of contact between a recombination protein and its target. The results are of special interest to molecular biologists because other work has suggested that IHF is a member of a previously unstudied class of specific DNA binding proteins that interact with the minor groove of DNA rather than the major groove.

Our investigation of the nature of the irreversible step in lambda recombination addresses a subject of key importance to understanding how recombination can be used to control differentiation. If, as widely believed, site-specific recombination switches genes on and off during development, then the cell must be able to distinguish "on" and "off". The lambda integration/exci-

sion cycle is the first model of such a directed switch that can be studied in vitro. Our results indicating that this directionality occurs at an early step in recombination points the way towards more precise localization of this distinction.

Proposed Course:

We are developing a new technique that will let us determine whether the binding of a protein to DNA is affected by supercoiling. Like the melting of DNA in response to supercoiling, such a possibility is supported by theoretical considerations but there is little experimental exploration of this possibility. Our innovation involves adapting the technique of chemical footprinting so as to permit probing of supercoiled DNA as well as the more traditional linear fragments. The technique, based on electrophoretic transfer of DNA fragments followed by detection via specific hybridization, should be sensitive to changes in position of the protein within the grooves of DNA and to changes in the strength of the protein-DNA interaction. The technique will first be applied to the interaction of recombination proteins with their target; other protein-DNA interactions of significance to molecular biology will also be studied.

The mutagenesis studies of the IHF binding site will be extended so as to further test the degree of flexibility at various positions in the DNA target for IHF. In this way we expect to define the features that IHF recognizes as it binds to its target. In addition, we will make multiple base changes in a given site to test whether the effect on recombination of these changes is additive or whether complete annihilation of a site leaves a residual level of recombination. We also plan to combine mutations so that two of the three IHF sites in attP are mutated. We want to know if particular pairs of sites work together or whether each binding site makes an independent contribution to the recombination potential of attP.

The experiment involving mismatched attachment sites is complete; it will be written up for publication.

Publications:

Peacock, S., Weissbach, H., and Nash, H. A.: In vitro regulation of phage λ cII gene expression by Escherichia coli integration host factor. Proc. Natl. Acad. Sci. USA 81: 6009-6013, 1984.

Lange-Gustafson, B. J. and Nash, H. A.: Purification and properties of Int-h, a variant protein involved in site-specific recombination of bacteriophage λ. J. Biol. Chem. 259: 12724-12732, 1984.

Craig, N. L. and Nash, H. A.: E. coli integration host factor binds to specific sites in DNA. Cell 39: 707-716, 1984.

Kitts, P., Richet, E., and Nash, H. A.: Lambda integrative recombination: Supercoiling, synapsis, and strand exchange. Cold Spring Harbor Symp.Quant. Biol. 49: 735-744, 1984.

Griffith, J. D. and Nash, H. A.: Genetic rearrangement of DNA induces knots with a unique topology: Implications for the mechanism of synapsis and crossing-over. Proc. Natl. Acad. Sci. USA, (in press).

Nash, H. A.: Lambda integrative recombination: Recombination proteins and their interaction with DNA. Presented at the 7th Annual Bristol-Myers Symposium on Cancer Research, New York, New York, 1985 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02228-01 LMB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Neurobiology of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. A. Nash	Medical Officer	LMB, NIMH
Others:	J. R. Whitaker, Jr.	Biologist	LMB, NIMH
	S. R. Haynes	Guest Researcher	LMG, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Molecular Genetics, NICHD

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.25	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have initiated a research program that will be devoted to the isolation of mutants affecting the nervous system of the fruit fly Drosophila melanogaster. The targets of this study in neurogenetics will be genes for known components of the fly's nervous system such as receptors for neurotransmitters and genes that underlie complex neurological processes whose components are not yet known. Initial work suggests that the former class may be approached by using formamidine insecticides as selective agents for mutations affecting aminergic transmission.

Objectives:

Genetic manipulation is a powerful tool for the analysis of complex biological processes. The isolation and characterization of mutants that affect a process has repeatedly been a major factor in elucidating its components and organization in organisms as diverse as E. coli and man. The same genetic techniques that have helped unravel pathways in intermediary metabolism, DNA replication, and embryonic development should be applicable to the study of behavior and the nervous system. Indeed, for the past fifteen years genetic studies of the fruit fly, Drosophila melanogaster, have turned up interesting and important mutations that affect sensory, motor, and integrative capacities of the organism. With the advent of contemporary genetic techniques, many of the Drosophila genes identified by these mutations are now being studied at the molecular level. In addition to its small genome and rapid generation time, Drosophila offers a special advantage for studying complex genetic loci. In the past three years techniques have been developed that readily permit the reintroduction of a cloned Drosophila gene back into the fly's genome, permitting the investigator to alter the gene at will and then assess its function in vivo. We are initiating a research program to investigate the neurobiology of Drosophila using genetics as the primary tool. We want to isolate mutants that will identify the genes for important components of the fly's nervous system such as ion-specific channels and receptors for neurotransmitters. In addition, we want to identify the genes that underlie complex neurological processes whose components are not yet known. The feasibility of such projects has already been demonstrated in other laboratories by the isolation of mutants that identify genes for components of sodium channel and genes which control circadian rhythmicity in the fruit fly.

Methods Employed:

In our initial experiments we plan to use the following general strategy. Flies will be treated with chemicals that act on a specific component of the nervous system and/or cause specific behaviors. Such chemicals could include psychopharmacologic agents, insecticides, and anesthetics. A population of mutagenized flies will be screened for an unusual response to these chemicals - e.g., an altered dose-response curve. Such mutants will then be inbred to isolate the affected gene. Chromosome mapping and dominance testing will then initiate a phase of detailed genetic characterization of the mutant genes.

Major Findings:

Our first efforts have been devoted to setting up an experimental framework for the project. We have established clonal cultures of wild-type Drosophila and several strains bearing the useful genetic markers. We have also explored a variety of routes for chronic and acute administration of chemicals to individual flies and to populations of flies; convenient feeding and topical application formats have been adopted. A small battery of semiquantitative tests of fly behavior has been established; the most subtle of these measures the fly's natural inclination to move against gravity, i.e., negative geotaxis. Finally, we have begun testing compounds of interest for their effect on fruit flies. Although the Drosophila nervous system has been reported to contain enkephalin-

immunoreactive material and specific receptors for opiates, we find no consistent behavioral consequence of either short or long term application of several opiates. We do not know if our route of administration is ineffective or if opiates affect behaviors that we have not tested. Preliminary studies with formamidines, a class of insecticides that act as octopamine agonists at a variety of invertebrate synapses, appear more promising.

Significance to Biomedical Research and the Program of the Institute:

Many components of the nervous system are difficult to isolate biochemically because they are present small amounts, are intermingled with other components in complex anatomic structures, lack facile assays, etc. The identification of the genes for such components should lead to useful tools to characterize these components at the biochemical and physiological levels. In addition, the cloned genes for these components can be used both to assess gene expression in the nervous system and to determine the nature of the gene product. To the extent that such genes are conserved in evolution, the identified genes could provide probes for these components in higher organisms.

Proposed Course:

We will pursue our investigation of formamidine insecticides as agents for selecting mutants affecting aminergic synaptic transmission. We will seek conditions where these compounds lead to gross disruption of behavior in a substantial proportion of wild-type Drosophila and then initiate screening for single-site mutants that show more normal behavior under these conditions. We also plan to initiate a search for Drosophila mutants that are altered in their sensitivity to the effects of general anesthetics. Such mutants would help identify the molecular and cellular sites where anesthetics act to disrupt consciousness and the perception of pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00934-13 LMB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Biochemical Basis of Narcotic Drug Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. A. Klee	Chief, Sec. on Regulatory Proteins	LMB, NIMH
Others:	W. F. Simonds	Medical Staff Fellow	LMB, NIMH
	B. Tocque	Visiting Fellow	LMB, NIMH
	G. Milligan	Visiting Fellow	LMB, NIMH
	R. C. Rice	Research Chemist	LC, NIADDK
	A. E. Jacobson	Research Chemist	LC, NIADDK
	T. Burke	Research Chemist	LC, NIADDK

COOPERATING UNITS *(if any)*

Laboratory of Neurophysiology, NINCDS; Laboratory of Chemistry, NIADDK; Laboratory of Developmental and Molecular Immunology, NICHHD and Laboratory of Biochemical Genetics, NIHLB

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Regulatory Proteins

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Progress in the past year has centered about the characterization and use of highly purified preparations of opiate receptors and the regulatory protein(s) which couple these receptors to adenylate cyclase. We have raised antibodies which specifically recognize opiate receptors in solution as well as in the solid state. These antibodies, after affinity purification should be useful for cloning, histochemical, and metabolic studies of the receptors.

The two components of the inhibitory regulatory protein preparation which we and others have purified from bovine brain are currently referred to as Ni and No. We have prepared and characterized antibodies that specifically recognize the α subunits of each (the β and γ subunits are apparently identical). The two proteins are difficult to separate from one another. We are therefore exploring the suitability of tissues other than brain as potential exclusive sources of only one or the other of these proteins. Liver, for example, contains no detectable No, but does have useful amounts of Ni. Bradykinin receptors in some cells stimulate phosphatidylinositol turnover in a process mediated by Ni or No. We have found that such receptors also stimulate GTPase and inhibit adenylate cyclase activities in NG108-15 neuroblastoma x glioma hybrid cell membranes. Our data suggest that bradykinin and opiate receptors may be coupled to different N proteins.

We have demonstrated the reconstitutive coupling of opiate receptors, Ni (or No) and adenylate cyclase in liposomes prepared from solutions of these proteins. Availability of a reconstitution assay should allow characterization of the activities of each of the purified components of the membrane signal transduction apparatus as well as identification of any as yet unknown components.

Other Investigators:

A. Spiegel	Medical Officer	MDB, NIADDK
P. Giershik	Visiting Fellow	MDB, NIADDK
R. Sekura	Research Chemist	LDMI, NICHHD
M. Nirenberg	Chief, Lab. of Biochemical Genetics	LBG, NIHLB
J. Barker	Chief, Lab. of Neurophysiology	LNP, NINCDS

Project Description and Major Findings:

We have for several years been engaged in the characterization of the interaction between opiate receptors and adenylate cyclase in a cultured neuronal cell line, NG108-15. The cell has a large number of opiate receptors which function to inhibit adenylate cyclase and thereby lower cAMP levels. In analogy to the addictive process, the cells become tolerant to, and dependent upon opiates after prolonged exposure. This adaptive response is due to a gradual increase in adenylate cyclase activity which serves to maintain normal cAMP levels in the continued presence of opiates. In the past year, we have made significant progress in our understanding of the detailed mechanism of some of these opiate actions. Work in this and other laboratories has shown that the cyclic nucleotide linked mechanism of opiate action is operative in brain as well as in the cultured cell system.

As a first step towards the purification and reconstitution of the cellular constituents involved in opiate action, we solubilized opiate receptors from several sources using the zwitterionic detergent, CHAPS. Receptors which reversibly bind opiate ligands with the appropriate specificity were isolated from membranes of NG108-15 cells, brain tissue (both rat and beef), and human placenta. Each of these receptor preparations behaves as a macromolecular complex of Stokes radius near 7 nm and contains protein as an essential constituent. In the past year Dr. Tocque has succeeded in effecting significant purification of this material by means of lectin and opiate affinity columns. The best preparations obtained to date have been purified approximately 2000 fold from crude membranes. This material is approximately 10% pure but may be used in reconstitution experiments which Dr. Tocque has recently been able to perform with somewhat less highly purified samples. In these experiments, a partially purified mixture of opiate receptors and adenylate cyclase is fortified with highly purified regulatory protein (Ni/No described below), and incorporated into phospholipid vesicles. Significant inhibition of adenylate cyclase activity by opiates is seen in the complete system but not in the absence of added regulatory protein. These experiments are a giant step forward in our ability to study the mechanism of signal transduction at the molecular level.

Opiate inhibition of adenylate cyclase activity is accompanied by, and may be mediated through stimulation of an associated, low Km, GTPase activity. In following up this observation, members of the section have studied the protein responsible for this GTPase activity, currently called Ni. This protein is one of several members of a family of GTP-binding proteins, the N proteins. Several signal transduction processes in cell membranes are mediated by receptor coupling to one of a family of GTP-binding proteins (N proteins). The N proteins are all closely related in structure and are composed of three subunits (α,

β, and γ). At least two such proteins couple receptors to adenylate cyclase: Ns is required for stimulatory receptor activity, and Ni (or perhaps No) for inhibitory receptors. The group has purified a mixture of Ni and No from bovine brain, but has so far been unable to separate the two in a useful way. Nonetheless, this preparation will restore opiate inhibition of adenylate cyclase and stimulation of low Km GTPase activity to membranes depleted of these activities by pertussis toxin-catalyzed ADP-ribosylation of Ni. In collaborative studies, they have prepared and characterized antibodies which specifically recognize the α subunits of Ni or No. (The β and γ subunits of the proteins are apparently identical.) These antibodies are being used to quantitate the tissue distribution of the two proteins after their electrophoretic separation. Western blots of membranes prepared from cells or tissues are analyzed by measuring the amount of I^{125}-protein A bound to them. Some tissues, such as liver and kidney, contain no detectable No but clearly contain Ni. The brain, on the other hand, is a very rich source of No and contains lesser, but still appreciable amounts of Ni. The functional roles of No and Ni are not yet completely understood. Because they are both substrates for pertussis toxin-catalyzed ADP-ribosylation this functional probe does not distinguish between the two. We are currently preparing pure Ni from liver for use in reconstitutive assays of N protein function to examine this question.

Rice, Jacobson and their colleagues prepared a series of opiate ligands containing alkylating functions so that they might serve as covalent affinity labels of the receptors. We studied the biological activities of these substances and found several of them to be uniquely selective, irreversible, ligands for either μ or δ receptor subtypes. The most useful of these compounds, to date, are fentanylisothiocyanate (FIT) which covalently binds to the (exclusively) δ receptors of NG108-15 membranes and super-FIT [(+)-3-Methylfentanylisothiocyanate]. Super FIT is 5-10x more potent than FIT, can be prepared as active and inactive enantiomers and shows much less non-specific binding than does FIT. Interestingly, these materials behave as agonists even when covalently bound. The radioactive materials bind specifically only to a 58,000 M_r glycoprotein. Because this binding is blocked by active opiates and not by their inactive enantiomers, we believe the protein to be the recognition subunit of the opiate receptor. It has been purified by Dr. Simonds by a combination of wheat germ lectin chromatography, antibody affinity chromatography and electrophoresis. Antibodies to FIT were prepared and purified by affinity chromatography. These were coupled to Sepharose and have been successfully exploited as an affinity matrix to effect a 200 fold purification of the labelled receptor subunits. The protein has been purified approximately 20,000 fold over starting membranes and appears to be homogeneous.

Dr. Simonds has recently increased the scale and efficiency of the purification procedure so that nanomole amounts of the protein are now available. He has raised antibodies against this protein and has shown that they effectively bind to unmodified opiate receptors in solution. Thus, the identification of the 58,000 Mr protein as the binding subunit of the opiate receptor has been confirmed. The antibodies also recognize opiate receptors transferred to nitrocellulose, and other matrices after electrophoretic separation. The sera are being purified by affinity chromatography in preparation for their use in

selection of those clones of expression vectors which carry the opiate receptor genome. We also anticipate that these antibodies will be useful histological stains and will facilitate study of the biosynthesis, processing, and breakdown of the receptors. Dr. Simonds has also found that the receptor can be efficiently converted to a much smaller protein by cleavage of peptide bonds following some of the methionine residues with cyanogen bromide. The fragment so formed, of Mr near 30,000 contains both the carbohydrate and opiate sites. This material is currently being purified for amino acid sequence studies. The data so obtained will be used to design oligonucleotide probes for gene cloning.

Significance to Biomedical Research and the Program of the Institute:

A major problem in biology is understanding the mechanism of signal-response coupling across cell membranes. Cells communicate with one another and with their environment largely through chemical messengers which are sensed by cell surface receptors and thereby elicit other chemical changes within the cell. The opiates, and related substances, are important transmitters of information in the nervous system. An understanding of how brain cells transmit and use such information is essential to the design of rational therapy for mental illness.

Proposed Course:

We plan to continue our efforts to understand the molecular basis of signal transduction, with particular emphasis on opiate receptor and related mechanisms. In the next year we hope to be able to use the purified protein components of the system in reconstitution experiments aimed at understanding the nature of the macromolecular interactions responsible for this kind of information transfer. In addition we plan to attempt the cloning of the opiate receptor gene in order to obtain complete amino acid sequence data and to provide tools for the study of the regulation of receptor levels. A third aspect of our work is the investigation of other N protein related receptor systems in order to better understand the mechanisms by which cells choose one or another transduction system.

Publications:

Rice, K. C., Jacobson, A. E., Burke, T. R., Bajwa, B. S., Streaty, R. A. and Klee, W. A.: Irreversible ligands with high selectivity towards Delta or Mu opiate receptor subtypes. Science 220: 314-316, 1983.

Hjelmeland, L. M., Klee, W. A. and Osborne, J. C., Jr.: A new class of nonionic detergents with a gluconamide polar group. Anal. Biochem. 130: 485-490, 1983.

Burke, T. R., Rice, K. C., Jacobson, A. E., Simonds, W. F. and Klee, W. A.: Tritiation of irreversible mu or delta specific opioid receptor affinity ligands to high specific activity. J. Rad. Lbl. Compd., 21: 693-702, 1983.

Klee, W. A., Koski, G., Tocque, B. and Simonds, W. F.: The mechanism of receptor mediated inhibition of adenylate cyclase. Adv. Cyclic Nucleotide Res., 17: 153-160, 1984.

Burke, T. R., Bajwa, B. S., Jacobson, A. E., Rice, K. C., Streaty, R. A. and Klee, W. A.: Synthesis and pharmacological properties of irreversible ligands specific for μ or δ opiate receptors. J. Med. Chem., 27: 1570-1574, 1984.

Lessor, R. A., Rice, K. C., Streaty, R. A., Klee, W. A., and Jacobson, A. E.: Irreversible ligands to opioid receptors based on biologically potent endoethenoripavines. Reversible binding of FIT to Mu and delta opioid receptors. Neuropeptides, 5: 229-232, 1984.

Klee, W. A., Milligan, G., Simonds, W. F., and Tocque, B.: The role of adenyl cyclase in opiate tolerance and dependence. In Shart, C. W. (Ed.): NIDA Research Monograph 54 Mechanisms of Tolerance and Dependence. National Institute of Drug Abuse, Rockville, MD, 1984, pp. 109-118.

Milligan, G. and Klee, W. A.: The inhibitory guanine nucleotide binding protein (Ni) purified from bovine brain is a high affinity GTPase. J. Biol. Chem. 260: 2057-2063, 1985.

Pines, M., Gierschik, P., Milligan, G., Klee, W., and Spiegel, A.: Antibodies against the C-terminal 5 kilodalton peptide of the alpha subunit of transducin cross react with the 40 but not the 39 kiladalton guanine nucleotide binding protein from brain. Proc. Natl. Acad. Sci. USA, 82: 4095-4099, 1985.

Simonds, W. F., Burke, T. R. Jr., Rice, K. C., Jacobson, A. E. and Klee, W. A.: Purification of the opiate receptor of NG108-15 neuroblastoma x glioma hybrid cells. Proc. Natl. Acad. Sci. USA, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01037-17 LMB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of the Cell Membrane in Cellular Organization: A Molecular Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David M. Neville, Jr.	Chief, Sec. on Biophy. Chem.	LMB, NIMH
Others:	Jon Marsh	Staff Fellow	LMB, NIMH
	Thomas Hudson	Staff Fellow	LMB, NIMH
	Hans Wellhoener	Guest Researcher	Hannover Univ.
	Daniel A. Vallera	Assistant Professor	U. of Minnesota
	J. H. Kersey	Prof. of Pediatrics	U. of Minnesota

COOPERATING UNITS *(if any)*

Minnesota Bone Marrow Transplantation Group, University of Minnesota, Minneapolis, Minnesota

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Biophysical Chemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.75	3.75	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of interaction, plasma membrane receptor mediated entry of proteins into the cell cytosol. These studies have been done by developing techniques to construct artificial protein conjugates containing the active fragment of a toxin and another receptor specific binding protein. Such artificial protein conjugates have value as a new class of pharmacologic reagents. Monoclonal antibody ricin conjugates or immunotoxins directed against human T cells effectively deplete these cells from donor bone marrow permitting bone marrow transplants apparently free from moderate to severe forms of graft versus host disease. This will provide a new treatment for leukemia, aplastic anemia and autoimmune diseases such as multiple sclerosis, Guillain Barre Syndrome, systemic lupus erythematosus and perhaps other diseases of the immune system such as acquired immunodeficiency syndrome. In addition, these reagents are useful for enzyme replacement therapy and in organ transplantation.

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. The major specific aim of the program is to understand how protein toxins after binding to membrane receptors are able to cross the plasma membrane and enter the cytosol compartment. These mechanisms are utilized by toxins and viruses and probably have an unknown physiologic counterpart.

A wide variety of proteins are capable of entering cells by receptor-mediated transport processes. Having gained entry these proteins are directed to specific cellular compartments where they exert either a physiological or pathological function.

In general it appears that only a discrete portion of these proteins contain the receptor binding activity which is involved prior to the entry process while another portion of the protein performs the intracellular function. Therefore, it is possible to split and reassemble two such proteins with a new combination of receptor entry specificity and intracellular function. Such proteins we call artificial hybrid proteins, and previous reports from this laboratory have suggested that such hybrids should have utility both as probes of entry processes and as a new class of pharmacologic reagents with tailor made receptor and therefore cell type specificity.

Major Findings:

1. Anti-T cell monoclonal antibody-ricin-conjugates or immunotoxins developed in this laboratory are proving to be efficient reagents for eliminating morbid graft-versus-host disease (GVHD) following allogeneic bone marrow transplantation for treatment of leukemia. Recent studies with collaborators at the University of Minnesota indicate that GVHD will cease to be a problem. However these new reagents and their treatment protocols will require more rigorous conditioning procedures to prevent graft rejection and leukemic relapses.

2. Enhanced efficiency of immunotoxins are achieved if immunotoxins are constructed to permit greater interactions of the toxin binding chains with cell membrane receptors. Increased efficiency allows the investigator to target cell subpopulations which are low in receptor number. This achievement broadens the panel of cellular determinants which can be utilized and widens the number of discrete cellular subpopulations which can either be spared or eliminated.

3. A kinetic study on the entry of diphtheria toxin (DT) into the cytosol compartment of cells reveals that entry is a quantal phenomenon. In a cell population exposed to diphtheria toxin the toxin enters cells via endocytotic vesicles at a uniform rate. However, entry to the cytosol compartment (where the toxin kills) appears to be a random event of rapid duration and involves many molecules of toxin. The most likely explanation is that the toxin contains a mechanism for opening a large pore or lysing the endocytotic vesicle.

4. The kinetics of tetanus intoxication can be followed in tissue culture using a neuronal glial hybrid cell line. This provides a highly standard tool for exploring the evoked release of neurotransmitters and their controlling mechanisms.

Significance to Biomedical Research and the Program of the Institute and

Proposed Course:

1. The apparant reduction in fatal and morbid cases of graft-versus-host-disease following immunotoxin treatment of donor marrow prior to bone marrow transplantation will increase the utility of this procedure and should extend its use to life threatening autoimmune diseases. Clinical trials are progressing to explore optimal ablative procedures to be used with this new adjunct to bone marrow transplantation.

2. The low entry efficiency of our first generation of antibody-toxin conjugates or immunotoxins indicates that we are not fully utilizing the toxin's inherent entry mechanisms. New approaches are being tested based on new insights into the entry mechanism.

3. The apparent discovery of how toxins cross the plasma membrane in phenomenological terms, "lysing or opening up the vesicle" is highly important in several respects. Because the mechanism is highly specific the toxin probably is utilizing a physiologic system. If this is the case, the physiologic function is unknown and this should be a fruitful area of investigation. Since the phenomenology appears settled the biochemistry should be made easier. These insights should help us to construct more efficient conjugates which could be used *in vivo* to eliminate unwanted cells.

4. The mechanisms which regulate and modulate neurotransmitter release are central to understanding how the nervous system records, recalls, and modifies memory. Tetanus toxin is a powerful probe for unraveling these processes which are basic to all mental activity. The biochemical identification of the neural substrate for tetanus toxin action should provide an enhanced understanding of these processes.

Publications:

Strong, R. C., Youle, R. J. and Vallera, D. A.: Elimination of clonogenic T-leukemic cells from human bone marrow using anti-M_r 65,000 protein immunotoxins. Cancer Res. 44: 3000-3005.

Esworthy, R. S. and Neville, D. M., Jr.: A comparative study of ricin and diphtheria toxin-antibody-conjugate kinetics of protein synthesis inactivation. J. Biol. Chem. 259: 11496-11504, 1984.

Hudson, T. H. and Neville, D. M., Jr.: Quantal entry of diphtheria toxin to the cytosol. J. Biol. Chem. 260: 2675-2680, 1985.

Filipovich, A. H., Vallera, D. A., Youle, R. J., Neville, D. M., Jr., and Kersey, J. H.: Ex vivo T cell depletion with immunotoxins in allogeneic bone marrow transplantation: The pilot clinical study for prevention of graft-versus-host disease. Transplantation Proc. 17: 442-444, 1985.

Neville, D. M., Jr.: Monoclonal antibody mediated drug delivery and antibody-toxin conjugates. In: Borchardt, R., Repta, A., and Stella, V. (Eds.): Directed Drug Delivery: A Multidisciplinary Problem. Humana Press, Inc. (in press).

Neville, D. M., Jr.: Immunotoxins: Current use and future prospects in bone marrow transplantation and cancer treatment. CRC Critical Review in Therapeutic Drug Carrier Systems. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01031-17 LNC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Conversion of Phenylalanine to Tyrosine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	Seymour Kaufman	Chief	LNC NIMH
	Thomas Nelson	Staff Fellow	LNC NIMH
	Desirazu Narasimha Rao	Visiting Fellow	LNC NIMH
	Michael Davis	Senior Staff Fellow	LNC NIMH
	Jennifer Tipper	Senior Staff Fellow	LNC NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously shown both in vitro and in vivo that the activity of rat liver phenylalanine hydroxylase can be activated by phosphorylation and deactivated by dephosphorylation. We have now demonstrated that the phosphorylation-mediated activation process can be modulated by both physiological concentrations of the enzyme's substrate, phenylalanine, and its coenzyme, tetrahydrobiopterin (BH_4): phenylalanine stimulates the phosphorylation reaction and BH_4 inhibits it. This regulatory mechanism would provide the organism with high phenylalanine hydroxylase activity when that activity is needed to dispose of elevated levels of phenylalanine that would be harmful to the developing brain.

We have found that rat kidney phenylalanine hydroxylase, when assayed with BH_4, is in a very high state of activation. As a result, the activity of this enzyme is much higher than previously realized. These results suggest that the kidney, previously believed to contribute only marginally to phenylalanine homeostasis in the whole organism, may play a much more important role in this process.

PHS 6040 (Rev. 1/84) GPO 904-917

Major Findings:

During the last year, our research on phenylalanine hydroxylase has focused on three aspects: structure of rat liver phenylalanine hydroxylase; regulation of rat liver phenylalanine hydroxylase; characterization of rat kidney phenylalanine hydroxylase.

Two features of the structure of rat liver phenylalanine hydroxylase were elucidated. Our earlier structural studies of the enzyme had established that the N-terminal amino acid is blocked. These studies, however, had stopped short of identifying either the terminal amino acid or the nature of the blocking group. We have now shown that the N-terminal amino acid is alanine and that the blocking group is an acetyl residue.

Evidence from our laboratory, as well as from others, had suggested that rat liver phenylalanine hydroxylase is made up of two different kinds of subunits. By analyzing the number and nature of the peptides produced when the enzyme is cleaved by cyanogen bromide (CNBr), we have demonstrated conclusively that our preparations of the hydroxylase (from Spague-Dawley rats) are composed of a single type of subunit. CNBr treatment, for example, cleaves the enzyme into only two peptides. There is only one kind of N-terminal amino acid, as well as the carboxyl-terminal amino acid. Furthermore, there is a single unique peptide sequence (extending to eight amino acids) beyond the CNBr cleavage point. These studies also established that the site of phosphorylation of the enzyme is on the N-terminal peptide that is obtained by CNBr cleavage.

A key aspect of the regulation of phenylalanine hydroxylase was discovered with the demonstration that the phosphorylation-mediated activation of phenylalanine hydroxylase is severely inhibited by physiological concentrations of the naturally-occurring coenzyme, tetrahydrobiopterin (BH_4), and modestly stimulated by the substrate, phenylalanine. Significantly, phenylalanine can overcome the inhibition by BH_4. This last effect represents a novel way in which this enzyme can be activated by its substrate.

It has been known that phenylalanine hydroxylase occurs in kidney as well as in liver. The kidney enzyme has been relatively neglected because it appeared to account for only 5% of the organism's phenylalanine hydroxylating capacity. During the last year, however, we have shown that this idea that the kidney enzyme makes only a minor contribution to phenylalanine metabolism is incorrect. When assayed with BH_4, instead of with a synthetic analogue of BH_4, the kidney hydroxylase probably accounts for 25-30% of the organism's total phenylalanine hydroxylase activity. We have purified the kidney enzyme to near homogeneity and have shown that it is in a super activated state compared to the liver enzyme. Furthermore, it cannot be further activated by any of the ways that are known to activate the liver enzyme. We are currently investigating the structural basis for this unusual activated state.

We have purified phenylalanine hydroxylase phosphatase 1800-fold from rat liver and found that it is a type 2A-phosphatase. The phosphatase had even greater activity toward phosphorylated tyrosine hydroxylase as toward phenylalanine hydroxylase. The phenylalanine hydroxylase-phosphatase has been shown to be a multimer of phosphorylase a phosphatase and a small phosphatase specific for phenylalanine hydroxylase and tyrosine hydroxylase.

Significance to Biomedical Research and Proposed Course of Project:

The demonstration that rat liver phenylalanine hydroxylase is composed of a single type of subunit makes it highly likely that the same is true for human liver phenylalanine hydroxylase. This finding simplifies the picture of the composition of the enzyme in PKU heterozygotes.

The finding that rat liver phenylalanine hydroxylase contains an N-acetylalanine residue as the N-terminal amino acid raises questions about whether the blocking group affects the functioning of the enzyme. We will explore ways to remove this group in order to determine whether it plays any role in the enzyme's catalytic function.

Our demonstration that phenylalanine and BH_4 work in opposition to each other in controlling the phosphorylation-activation of phenylalanine hydroxylase represents a novel way in which phenylalanine can activate the enzyme. This appears to be an important step in the redundant regulation of the activity of the hydroxylase by its substrate. Since this mechanism of activation of phenylalanine hydroxylase would lead to the more rapid disposition of a bolus of phenylalanine, it seems likely that these effects of phenylalanine function to protect the fetal and neonatal brain from the known deleterious effects of elevated phenylalanine levels. We are attempting to determine whether this control mechanism operates in vivo.

The finding that kidney phenylalanine hydroxylase is in a highly activated state requires a reassessment of the role of the kidney enzyme in the overall phenylalanine homeostasis in the organism. Specifically, it raises a question about whether the kidney enzyme helps to maintain normal blood phenylalanine levels following the rise in the level of this amino acid in the blood that occurs after the ingestion of a protein containing meal. In addition to addressing these questions, we want to examine the structural basis for the highly activated state of the kidney enzyme.

The phosphatase experiments have provided evidence that phenylalanine hydroxylase phosphatase and phosphorylase a are co-regulated by a common phosphatase as well as by a common protein kinase. Further study of the enzyme could lead to additional insights into the interrelationships between carbohydrate metabolism and neurotransmitter biosynthesis.

Publications:

1. Phillips, R. S., Parniak, M. A., and Kaufman, S.: Spectroscopic investigation of ligand interaction with hepatic phenylalanine hydroxylase: Evidence for a conformational change. Biochem. 23: 3836-3842, 1984.

4. Abita, J.-P., Parniak, M., and Kaufman, S.: The activation of rat liver phenylalanine hydroxylase by limited proteolysis, lysolecithin and tocopherol phosphate: Changes in conformation and catalytic properties. J. Biol. Chem. 259: 14560-14566, 1984.

5. Iwaki, M., Parniak, M. A., and Kaufman, S.: Studies on the primary structure of rat liver phenylalanine hydroxylase. Biochem. and Biophys. Res. Comm. 126: 922-932, 1985.

6. Kaufman, S.: Enzyme control by phosphorylation: Aromatic amino acid hydroxylases. The Enzymes, 1985. IN PRESS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01032-17 LNC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biosynthesis of Catecholamines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	Seymour Kaufman	Chief	LNC NIMH
	Kenneth Davis	Senior Staff Fellow	LNC NIMH
	Thomas Nelson	Staff Fellow	LNC NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tyrosine hydroxylase catalyzes the rate-limiting step in the biosynthesis of the neurotransmitters dopamine and norepinephrine. We are purifying the enzyme from bovine striatal tissue.

Project Description:

The objective of this research project is the detailed description of the hydroxylation reactions that are involved in the biosynthesis of the neurotransmitters, dopamine and norepinephrine. Recently, we have been focusing on attempts to purify tyrosine hydroxylase from brain in order to further clarify the molecular mechanism of activation of this enzyme by phosphorylation.

Major Findings:

We are trying to devise a high-yield, large-scale purification of the enzyme from bovine striatal tissue. In view of its great potential, we are exploring the possibility of raising monoclonal antibodies to the the enzyme that would be used for the reversible and specific immuno-adsorption of the enzyme from crude tissue extracts.

Significance to Biomedical Research Proposed Course of Project:

Tyrosine hydroxylase catalyzes the rate-limiting step in the reaction sequence leading from tyrosine to dopamine and norepinephrine. Any factor that can alter the activity of this enzyme can change tissue levels of dopamine and norepinephrine. One of those factors is activation of the enzyme by phosphorylation. We plan to continue to try to purify the enzyme from brain tissue.

Publication:

1. Kaufman, S.: Regulatory properties of phenylalanine, tyrosine and tryptophan hydroxylases. Biochem. Soc. Transactions. 1985. IN PRESS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01038-17 LNC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phenylketonuria and Other Diseases Caused by Defects in Biopterin-Dependent Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	Seymour Kaufman	Chief	LNC NIMH
	Sheldon Milstien	Research Chemist	LNC NIMH
	Joseph Muenzer	Medical Staff Fellow	HGB NICHD

COOPERATING UNITS *(if any)*

Human Genetics Branch
National Institute of Child Health and Human Development, NIH
Bethesda, Maryland 20205

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

When a child with hyperphenylalaninemia (HPA) due to a defect in BH_4 synthesis was treated with subcutaneous injections of BH_4, only about 10% of the oral dose was required to get a comparable neurological and biochemical response.

A variant form of HPA due to a defect of BH_4 has been demonstrated in which the defect is restricted to the periphery. In order to evaluate the need for BH_4 replacement therapy in HPA due to BH_4 deficiency, pterin and biogenic amine metabolite levels in CSF must be determined.

Project Description:

In 1975, cases of hyperphenylalaninemia were reported in which neurological disorders persist despite dietary control of phenylalanine blood levels. Subsequently, variant forms of phenylketonuria (PKU) or hyperphenylalaninemia were described by our laboratory in which the defect in the phenylalanine hydroxylase system is not in phenylalanine hydroxylase, itself, as it is in classic PKU, but rather in dihydropteridine reductase or in an enzyme involved in the biosynthesis of tetrahydrobiopterin (BH_4). Dihydropteridine reductase functions to maintain BH_4 in its functional tetrahydro form while BH_4 is an essential coenzyme. Both of these variants are therefore characterized by a marked deficiency of BH_4. Since, as previous work in this laboratory had shown, this pterin is an essential coenzyme not only for phenylalanine hydroxylase, but also for tyrosine and tryptophan hydroxylases, patients lacking BH_4 suffer from defects in the synthesis of the neurotransmitters, dopamine, norepinephrine, epinephrine and serotonin in both the peripheral and central nervous sytems, as well as from an impaired ability to hydroxylate phenylalanine in the liver. Indeed, to our knowledge, these patients are the only population presently available whose neurological dysfunctions can unequivocally be attributed to a genetic defect in biogenic monoamine synthesis which does not appear to involve irreversible cell loss. These patients might therefore be considered as models for other nondegenerative neurological diseases, the etiology of which is believed to involve aberrations in biogenic monoamine metabolism.

Major Findings:

We have previously demonstrated that, contrary to the accepted view, the oral administration of relatively large amounts of BH_4, 10-20mg/kg/day, leads to very significant entry of the administered pterin into the brain. As a result, the central deficits in biogenic amines can be largely corrected.

One of the problems with this form of treatment is that it is extremely expensive. At the present price of BH_4 of about $140/gram, treatment of a 20 kg child would cost $15,000/yr. Since this treatment would have to be continued throughout life, this cost would rise to $45,000/yr for a 60 kg adult.

We have been exploring various routes of administration of BH_4 to a child with hyperphenylalaninemia due to a defect in BH_4 synthesis and have found that subcutaneous injection of the BH_4 is about 10 times more effective than an oral dose in keeping blood levels of BH_4 within the necessary range. This result suggested that only 10% of the previously established oral dose would be required if the subcutaneous route were used.

Based on these results we have maintained this patient on this lower, subcutaneous dose for more than six months. To date, the patient's biochemical and neurological response to the subcutaneous administration of the BH_4 have been comparable to those seen after oral administration.

We have described two patients with hyperphenylalaninemia due to a block in BH_4 synthesis whose metabolic lesions appear to be restricted to peripheral tissues. Their CSF levels of BH_4 and biogenic amine metabolites are within the normal range and, consonant with our biochemical findings, these children are developing normally without replacement therapy. These results indicate that therapy of this variant form of PKU must be based on a demonstrated abnormality in CSF rather than in urine.

Significance to Biomedical Research and Proposed Course:

Our demonstration that, when administered subcutaneously, only 1/10th the dose of BH_4 is needed compared to the oral route, has important implications for the long-term treatment with BH_4 of any condition due to a deficit in tissue levels of BH_4. We plan to continue our investigation of the effectiveness of various routes of BH_4 administration to BH_4-deficient patients.

The characterization of a form of BH_4 deficiency in which the defect is restricted to the periphery will require a major change in the criteria used to decide whether BH_4 replacement therapy is to be initiated in any patient with hyperphenylalaninemia due to a BH_4 deficiency. Our results indicate that the decision to treat must be based on a demonstrable BH_4 deficiency in CSF rather than in urine.

Publications:

1. Hoganson, G., Berlow, S., Kaufman, S., Milstien, S., Schuett, V., Matalon, R., Naylor, E., and Seifert, W.: Biopterin synthesis defects: Problems in diagnosis. Pediatrics. 74: 1004-1011, 1984.

2. Kaufman, S.: Hyperphenylalaninemia caused by defects in biopterin metabolism. J. Inherited Metabolic Dis. 1985. IN PRESS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01039-17 LNC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pteridine Biosynthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	Sheldon Milstien	Research Chemist	LNC NIMH
	Seymour Kaufman	Chief	LNC NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tetrahydrobiopterin (BH_4) biosynthesis has been shown to proceed through tetrahydro intermediates. The first tetrahydro intermediate has been shown to be 6-pyruvoyl-tetrahydropterin. The enzyme catalyzing the formation of this intermediate, 6-pyruvoyl-tetrahydropterin synthase, has been purified from brain and liver. Sepiapterin reductase catalyzes the last reaction in BH_4 biosynthesis.

Project Description:

Tetrahydrobiopterin (BH_4) is the coenzyme required for the hydroxylation of phenylalanine, tyrosine and tryptophan. Children who have a genetic defect in BH_4 biosynthesis have phenylketonuria due to their inability to metabolize phenylalanine and severe neurological problems as a result of a lack of those neurotransmitters which are produced from the hydroxylated amino acids. The BH_4 biosynthetic pathway has not yet been completely elucidated. The genetic defect(s) in such children has not yet been identified, except in one case of a child who is missing the first enzyme in the pathway.

Major Findings:

The biosynthesis of tetrahydrobiopterin (BH_4) from guanosine triphosphate starts with the formation of dihydroneopterin triphosphate, a reaction catalyzed by the enzyme, GTP-cyclohydrolase. The subsequent reactions in the pathway have been shown to proceed through tetrahydropterin intermediates. Dihydroneopterin triphosphate is converted to 6-pyruvoyltetrahydropterin in a reaction involving the elimination of the triphosphate group. The conversion of 6-pyruvoyl tetrahydropterin to BH_4 requires the reduction of two carbonyl groups.

It appears that in the liver, the enzyme sepiapterin reductase may catalyze the direct reduction of this intermediate to BH_4. In the brain, however, there is another reductase which can form 6-lactoyl-tetrahydropterin as an intermediate, which then is reduced to BH_4 by sepiapterin reductase. This finding remains the only difference so far discovered between the BH_4 biosynthetic pathways in the periphery vs. the CNS.

Significance to Biomedical Research and Proposed Course:

A simple purification scheme for sepiapterin reductase has been developed. This should allow us to separate and identify the remaining unknown reactions in BH_4 biosynthesis. Antibodies to sepiapterin reductase are being prepared with the goal of obtaining a cDNA probe to screen genetic carriers of BH_4 deficiency. 6-Pyruvoyl-tetrahydropterin synthase from brain and liver have also been purified and will be cloned. Recent work from other laboratories indicate that this may be the missing enzyme in children with BH_4 deficiency.

Publications:

1. Milstien, S., and Kaufman, S.: The role of sepiapterin reductase in tetrahydrobiopterin biosynthesis. In Pfleiderer, W., Wacter, H., Curtius, H. C. (Eds.): Biochemical and Clinical Aspects of Pteridines, Vol. 3, Walter de Gruyter & Co., Berlin, 1984, 53-60 pp.

2. Milstien, S., and Kaufman, S.: Biosynthesis of tetrahydrobiopterin: Conversion of dihydrotriphosphate to tetrahydropterin intermediates. Biochem. Biophys. Res. Comm. 128, 1099-1107, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01040-01 LNC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of the Pterin-Dependent Hydroyxlases Ancillary Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	Seymour Kaufman	Chief	LNC NIMH
	Sheldon Milstien	Research Chemist	LNC NIMH
	D. N. Rao	Visiting Fellow	LNC NIMH
	Fred D. Ledley	Research Associate	Baylor University
	Savio L.C. Woo	Professor	Baylor University

COOPERATING UNITS *(if any)*

Baylor University, School of Medicine, Houston, Texas

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Putative clones of human liver phenylalanine hydroxylase and dihydropteridine reductase (DHPR) have been isolated and are being characterized.

Project Description:

Two of the major goals of this project are: 1) obtain large amounts of the pure enzymes through the use of recombinant DNA techniques, as a supplement to attempts to obtaining them by the techniques of protein fractionation. There are many essential structural studies that can only be carried out if large amounts of the pure proteins are available. In addition, the availability of very much larger quantities of enzymes such as phenylalanine hydroxylase would be the first step in the exploration of their use in enzyme replacement therapy for certain genetic diseases; 2) isolation of the genes for these enzymes to study the regulation of their expression in various cell types. In addition, they can be used as probes in the prenatal diagnosis of genetic diseases caused by the lack of the enzymes. Finally, the availability of these genes would allow a study of the use of the genes as therapy for genetic diseases caused by a lack of the defective gene.

Major Findings:

We have used specific antibodies to screen human liver cDNA libraries for clones of both phenylalanine hydroxylase and DPHR. Both of these screening projects have yielded encouraging preliminary results, i.e., clones have been detected that react with the specific antibodies.

Significance to Biomedical Research and Proposed Course:

We are continuing our studies aimed at more fully characterizing these clones. In the case of the putative clones for phenylalanine hydroxylase, these studies involve cutting the DNA with a series of restriction enzymes; in the case of the putative clones for DHPR, these studies involve a determination of partial amino acid sequences of pure DHPR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00981-20 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanical, Thermal and Optical Signs of Excitation in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ichiji Tasaki	Chief, Unit of Neurobiology	LNP, NIMH
Others:	Toshio Nakaye	Visiting Fellow	LNP, NIMH

COOPERATING UNITS (if any)

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SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We continued and expanded recording of non-electrical signs of excitation processes in the nervous system. We found that the turbidity of the dorsal column of the frog spinal cord increases in response to electric stimuli applied to the dorsal roots. We obtained records showing a rise of the temperature of the cord evoked by afferent impulses. Next, we demonstrated that the dendrites of the amacrine and ganglion cells in the frog retina respond to light stimuli with slow contractions. Finally, we analyzed the sequence of light-induced events in the retina by taking heat production as an index. Attempts are now being made to elucidate the nature of these newly discovered mechanical and thermal phenomena in the nervous system.

Objectives:

The objective of the present research is to describe the process of excitation in the nervous system in terms of physical changes of the structure of the cell surface. Changes in the membrane conductance are known to be associated with movements of water molecules, which could be detected by mechanical and optical means. Fluxes of Ca-ions, which are involved in many aspects of physiological events in the nervous system, are known to evoke mechanical and thermal changes in the cells. We believe that synaptic and sensory excitation processes also involve mechanical and thermal changes in neurons and receptors.

Methods Employed:

(1) Sensitive piezoelectric sensors constructed with ceramic material were employed, (2) A thermal detector was constructed by using polyvinylidene fluoride film (which was a gift of Kureha Chemical Co. in Tokyo). The time-resolution and the sensitivity of this thermal detector were found to be far better than those employed by previous investigators. (3) A bifurcated light-guide was used for detection of turbidity or absorbance changes in the nervous system: one branch of the Y-shaped light-guide was used to lead monochromatic light to the nervous tissue, and the other branch was employed to detect changes in the light reflected or scattered by the tissue. (4) The frog spinal cord and retina were used in studies of synaptic and sensory mechanisms. In the Marine Biological Laboratory in Woods Hole, the nervous system of the squid was employed because of its structural simplicity.

Major Findings:

(1) By applying the bifurcated light-guide to a cut surface of the frog spinal cord, optical signals were recorded representing a rise in the turbidity of the grey matter evoked by the arrival of afferent impulses. The time-course of the optical signal was reminiscent of the dorsal root potential. The possibility that depolarization of the afferent terminals may give rise to a relatively large structural change is now under study. A sign of reduction of cytochromes in the spinal cord following the arrival of afferent impulses was observed; however, it was difficult to separate absorbance signals from the well-established turbidity signals.

(2) Using the piezoelectric sensor, the dendrites of the amacrine cells in the frog retina were found to "contract" in response to a brief pulse of light stimulus. A large contractile response of the dendrites of the ganglion cell was demonstrated by stimulating the optic nerve antidromically. The significance of the contractile responses of the dendrites in not clear at present.

(3) By using the sensitive thermal detector constructed in the laboratory, the heat evolved by the dark-adapted retina in response to a brief light pulse was found to consist of the following three components. (i) The component generated by direct conversion of the radiant energy into heat, (ii) the receptor heat that represents the occurence of an exothermic reaction triggered by the light pulse, and (iii) the heat generated by the post-synaptic elements in the retina. The characteristics of these three components of the heat signal were extensively analyzed.

Significance to Biomedical Reserch and to the Program of the Institute:

The phenomenon of swelling of nerve fibers and cells during excitation, which was discovered in this laboratory few years ago, indicates that the movement of water plays an important role in the process of excitation. We have demonstrated that the excitation process taking place at synapses and sensory endings is accompanied by readily detectable changes in the optical, mechanical and thermal properties of the nervous tissues. There is little doubt that these changes are at the base of the normal function of the nervous system. In the past, physiologists relied on devices for measuring electrical properties of nerve fibers and cells in studying the function of the nervous system. We believe that studies on non-electrical signs of excitation lead us to a better understanding of the normal, as well as abnormal, function of the nervous system.

Proposed Course of the Project:

Since the relationship between the thermal and mechanical responses of the retina has not been clarified, we are planning to investigate how these two different signs of excitation are related to each other. Next, we are hoping to be able to complete our optical and thermal study of the frog spinal cord.

Publications:

Tasaki, I.: Electrical, mechanical and thermal signs of nervous activities. A keynote address delivered in 62nd Annual Meeting of Japanese Physiological Soc. (in press).

Tasaki, I. and Byrne, P. M.: Mechanical changes in the amphibian spinal cord produced by afferent volleys of nerve impulses. Brain Res. 301: 265-272, 1984.

Tasaki, I. and Nakaye, T.: Heat generated by the dark-adapted squid retina in response to light pulses. Science 227: 654-655, 1985.

Tasaki, I., Nakaye, T. and Byrne, P.M.: Rapid swelling of neurons during synaptic transmission in the bullfrog sympathetic ganglion. Brain Res. 331: 363-365, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01081-15 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cerebral Control of Voluntary Movement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. V. Evarts	Chief	LNP, NIMH
Others:	S. Pullman	Medical Staff Fellow	LNP, NIMH
	R. Watts	Medical Staff Fellow	LNP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.60	2.60	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major goals of the project are to understand the mechanisms of information processing occurring within somatic sensorimotor cortical columns and the basal ganglia, and to determine how this relates to motor behavior. Extracellular single neuron recording techniques in awake, behaving monkeys are used to study neural mechanisms involved in the cerebral control of movement. Motor cortex responses to cerebellar stimulation, obtained via chronically implanted electrodes in the brachium conjunctivum, were recorded using microelectrodes in the primary motor cortex. Similarly, somatic sensorimotor cortex responses to peripheral mechanical stimulation, generated by controlled wrist flexion and extension using an electronically controlled torque motor, were recorded using microelectrodes in the primary motor cortex and somatic primary sensory cortex. Input-output relations of the somatic sensorimotor cortical columns were studied by investigating the effects these types of afferents to the cerebrum have on neurons in the different layers of the columns. This cortical activity in response to sensory signals can be contrasted with the properties of sensory responsive cells in the putamen and globus pallidus of the basal ganglia. A new and more versatile computer program was developed to sort and analyze event-related neural activity.

Objectives:

The input-output organization of the primary motor cortex (MI) may be viewed physiologically from the perspective of the cortical column. This vertical arrangement of histologically distinct cellular layers is the elementary functional unit of the cortex where afferent information arriving predominantly in the superficial layers (layer II and layer III) is ultimately expressed through pyramidal tract neurons (PTNs), all of which are in layer V. The purpose of this experiment was to study information processing within the cortical column by investigating how input to MI differentially affects activity in the superficial neurons and PTNs, and how this is related to motor behavior.

A large portion of the afferent information to MI arises from the interpositus and dentate nuclei of the cerebellum, and is relayed to the cortex via the ventrolateral nuclear complex of the thalamus (VL). Another large portion of input to MI arises from peripheral receptors. These inputs may also be relayed, in part, through VL. In the cat, intracellular recordings of PTNs in MI have revealed monosynaptic excitatory postsynaptic potentials (EPSPs) in response to electrical stimulation of VL. Large monosynaptic EPSPs were present in PTNs with relatively large axons, inferred from the short latencies of their antidromic responses, whereas PTNs with smaller axons had EPSPs that were disynaptic and lower in amplitude. Despite those studies, little information is available regarding the responsiveness of neurons in cortical layer III, where the terminal arborizations of cerebello-thalamocortical fibers are concentrated. This was due to the difficulty in obtaining satisfactory intracellular recordings from these smaller superficial neurons. The goal of the present study was to determine the responsiveness of the more superficially situated motor cortex neurons as compared to more deeply situated PTNs, in an awake monkey not trained to perform a motor task. Similar questions arise regarding the primary somatic sensory cortex (SI) and these were investigated in the same manner as for MI.

Methods Employed:

Extracellular single unit responses were isolated in precentral (primary) motor cortex (MI) and somatic sensory cortex (SI) using glass-coated platinum-iridium or Elgiloy microelectrodes in two awake rhesus monkeys (*Macaca mulatta*). The monkeys sat quietly in a primate chair and were not trained to execute specific movements. The recording microelectrodes were inserted transdurally within a stainless steel chamber centered stereotaxically over the forelimb region of the left MI cortex. Data obtained from each isolated unit included its location and depth within the cortex and its responsivess to cerebellar stimulation at different stimulus intensities.

Stimulation of cerebellar inputs to MI was carried out with bipolar electrodes chronically implanted near the origin of the right brachium conjunctivum, that delivered 0.2 msec constant current stimuli from 0.3 to 1.5 mA. PTNs were identified by their antidromic responses to electrical stimulation of the left medullary pyramid using bipolar electrodes chronically implanted just rostral to the pyramidal decussation. All putative PTNs were tested by the spike collision method and none were accepted unless they exhibited the appropriate collision properties.

Cerebellar-evoked cortical field potentials were sequentially recorded at 0.5 mm intervals from the surface of the dura vertically down through the cortex, and were used as a physiological marker to determine laminae in which neurons most responsive to cerebellar input were located. The point of sign reversal of the field potenial in relation to depth was used to make this determination.

For peripheral stimulation, ramp flexor and extensor displacements of the right wrist of up to 15 degrees and of 50 msec duration were produced by a DC torque motor. Alternation between wrist flexion and extension occurred every 2 seconds, with the wrist held in either position during the 2 sec interval.

Electrolytic marking lesions were made five days prior to the end of the experiment via the recording microelectrodes in the cortex near units with prominent responses to cerebellar stimulation. These were used to identify the cortical layer most responsive to cerebellar input. Similar lesions made via the chronically implanted electrodes in the brachium conjunctivum and medullary pyramid were used to confirm their respective positions. The animals were then deeply anesthetized with pentobarbital and the brains perfused with 10% formol saline. Brain sections were stained for Nissl substance with 0.25% thionin, and the locations of the marking lesions and electrode tracts were examined histologically.

In the study of the basal ganglia, monkeys were trained to make repeated self-paced movements for a juice reward. The delivery of the reward was preceded by the click of a solenoid valve which came to be a trigger for movements to consume the juice. Extracellular microelectrode recordings in putamen yielded a number of tonic neurons that were unrelated to body movements. However, in raster displays of cell discharge aligned on the occurrence of the solenoid click that signaled reward, it was apparent that there was a greatly increased probability of impulse occurrence approximately 60 msec following the click. This observation led to an examination of the responsiveness of tonically active neurons in three behavioral conditions: (a) self-paced movement in which a series of elbow movements resulted in a solenoid click and a juice reward; (b) free-reward, in which click and juice occurred at regular intervals (every 6 sec) with arm position fixed; (c) no-reward which was similar to free-reward except that the tube conveying the juice was occluded so that the solenoid click was no longer followed by juice.

Single unit activity (spike data) and kinematic parameters were collected on-line with a PDP 11/03 computer and an ANALOGIC Data 6000 Signal Analyzer. The data were analyzed off-line with PDP 11/23 and 11/34 computers, as well as with the Data 6000 Signal Analyzer programs. The analysis program used on the PDP 11 systems is described in detail below.

Major Findings:

1. Motor cortex responses to cerebellar stimulation.

Data were obtained on 494 superficial (layers II and III) neurons and 158 PTNs in MI. With constant current stimuli between 0.3 and 0.5 mA for 0.2 msec to the brachium conjunctivum, 141 (29%) of the superficial neurons and 5 (3%) of the

PTNs were found to respond to the stimulus. The typical evoked response exhibited by the superficial neurons consisted of a single spike of activity with a latency of 3 msec occuring in over 60% of trials. This was then followed by an inhibitory period of 30 to 40 msec before resumption of spontaneous activity. The few responsive PTNs, however, showed a slightly more dispersed response and exhibited longer latencies, usually from 3 to 8 msec. Increasing the stimulus intensity to 1.0 mA often evoked a bimodal response in superficial neurons, with the first spike occuring at a latency as fast as 2.5 msec and the second at latencies from 6 to 8 msec.

The initial deflection of the cerebellar-evoked cortical field potential was recorded at a latency of 1.6 msec. The potential was surface-positive and turned negative within the cortex. Laminar analysis revealed that the sign reversal occured about 1.5 mm from the surface of the cortex. This was the general region in which cerebellar responsive units were first obtained with the microelectrode moving vertically down through the cortex, and these could be seen superimposed on the field potential. Electrolytic marking lesions made at this depth were found, upon subsequent histological analysis, to be in cortical layer III.

In summary, it was found that responses to stimulation of the brachium conjunctivum (the cerebellar output) were approximately ten times more prominent in cells of cortical layers II and III than in PTNs (layer V). The greater prevalence of cerebellar-evoked unit responses in layer III is consistent with anatomical data showing a concentration of thalamocortical termination in layer III. While this does not contradict previous anatomical and physiological evidence indicating the existence of monosynaptic thalamic inputs to PTNs, the present results show the relatively greater strength of inputs to layer III neurons under the conditions of relaxed immobility that prevailed in this experiment.

2. Primary somatic sensorimotor cortex responses to peripheral input.

In both MI and SI, excitatory responses to peripheral mechanical stimuli were more prominent in superficial non-PTNs (layer III cells in MI and layer III and IV cells in SI) than in PTNs. Inhibitory responses were not systematically analyzed. Of a total of 226 non-PTNs recorded in MI, 78 (35%) were activated by wrist displacement whereas only 7 (12%) of 56 PTNs were responsive to wrist displacement. In SI, 260 (74%) of 352 non-PTNs were responsive to wrist displacement and 9 (40%) of 22 PTNs responded.

Responses in MI and SI could be grouped into 3 general categories: phasic, mixed phasic/tonic, and purely tonic. Phasic units responded at short latency after the onset of the ramp producing wrist displacement with an increase in discharge frequency, after which they rapidly returned to their prestimulus firing rate. Mixed units exhibited a phasic response to the ramp but also maintained an increased firing frequency below peak but above the prestimulus discharge rate as long as the wrist was held flexed or extended. Purely tonic units responded at longer latencies but had a sustained, lower level increase in firing rate as long as the wrist was maintained in a flexed or extended position.

Of the 78 responsive MI non-PTNs, 68 were of the purely phasic type (87%), 6 were of the mixed phasic/tonic type (8%), and 4 were of the purely tonic type (5%). In SI, the responses of the 260 non-PTNs consisted of 195 phasic (75%),

44 mixed phasic/tonic (17%), and 21 purely tonic (8%). Of the 7 responsive MI PTNs, 2 were of the phasic type, 3 were of the mixed type, and 2 were of the purely tonic type. The 9 SI PTN responses consisted of 3 phasic, 5 mixed, and 1 purely tonic.

In the analysis of response latency characteristics, the phasic and mixed phasic/tonic units were grouped together. The median response latencies (MRL) of the phasic and mixed MI and SI non-PTNs were the same at 16 msec, with ranges of 10-46 msec. The MRL of the 5 phasic and mixed MI PTNs was 26 msec (range 22-31), while in SI it was 20 msec (range 13-22) for the phasic and mixed PTNs.

These data indicate that in awake monkeys, not performing a motor task, PTNs are significantly less responsive to somatosensory stimulation than are more superficially situated non-PTNs in both MI and SI. The proportion of responsive non-PTNs to PTNs was 3:1 in MI and almost 2:1 in SI. Also, the response latencies of MI and SI phasic and mixed non-PTNs were significantly shorter than those of PTNs. This implies that at least some processing of afferent information occurs within the sensorimotor cortical column before effecting a change in activity of the output neurons (PTNs). These findings parallel results on differences between superficial and deep MI neurons in responsiveness to cerebellar-evoked responses (see above). A second major implication of these data concerns the pathway by which shortlatency somatosensory information reaches MI. Many have held that such information must first synapse in SI, and that corticocortical fibers from SI to MI would provide the short-latency somatosensory input. Our data argue against this since the fastest response latencies of both MI and SI were identical at 10 msec.

3. Basal ganglia responses to sensory inputs.

There was no apparent differences between solenoid-evoked activity in the self-paced movement condition versus the free-reward condition (see Methods, above), showing that arm movements prior to the solenoid click made little difference in the tonic neuron response. Lack of reward, however, led to disappearance of tonic cell responses, indicating the "set"-dependent nature of the sensory response.

One hundred seventy-four putamen cells with tonic discharge were examined, and 63% of these cells exhibited characteristic responses to the solenoid click. Typically, the solenoid click that was a cue for reward was followed by one impulse at a latency of about 60 msec, and this impulse was followed by a slightly lengthened interspike interval prior the resumption of tonic activity. Though related to the "set" of the monkey to consume reward, as indicated by the lack of response when the solenoid click was not followed by reward, the responses in the tonic neurons were not related to licking movements *per se*. Other putamen neurons had bursts of discharge with each of the series of self-paced arm movements or licking movements, but these cells lacked tonic discharge. In contrast, the tonic neurons responded to the solenoid clicks with single impulses well in advance of the first in the sequence of licking movements, and showed no apparent relation to the subsequent successive licks.

Tonically discharging putamen cells with set-sependent responses were observed at a number of loci within the putamen, but did not appear to be clustered in any particular somatotopic locus (e.g., orofacial, arm or leg areas).

Neuronal activity in the globus pallidus was recorded in the same three click-reward contingencies and click-responses were observed in a number of globus pallidus neurons. But these responses, pauses in neuronal activity, disappeared soon after the no-reward sequence started. Sixty globus pallidus cells showed set-dependent click responses and 39 were decreases of discharge.

4. Neurophysiological analysis program.

An interactive computer program for analysis of physiological data acquired was developed within the past year by K. Arrington. It allows the user to interact with neurophysiological data in ways not previously possible and was essential to the completion of the present project.

The program runs on a Plessey (DEC) 11/23 equipped with a matrox video display board, video monitor, and at least 5000 blocks of free disk space for work and scratch files. The terminal screen is divided into two parts. The top half of the screen displays program information as specified by the user. The bottom half of the screen scrolls up as the user interacts with the program. The matrox video graphics display is used to visually correlate pulse data, event codes, and analog data as they vary in time. Pulse data is primarily single-unit neuronal activity although mutiunit or muscle (EMG) activity can also be used. Event codes indicate the time of occurrence of signals or a behavior. And analog data is primarily limb position or force and their derivatives.

The program first looks through a data file for sequences of event codes (or code sets) which satisfies the user's specification. The data may then be sorted in any one of several ways: e.g., on the basis of the time that elapses between any two events or the number of pulses (single-unit discharges) that occur over a specified time interval. Four of these sorts are performed solely on the pulse and code data. The others are performed by determining some characteristic in the original or processed (e.g., differentiated) analog data, such as the peak acceleration of the limb during a passively imposed movement.

The user specifies the window, in milliseconds, which is to be graphically displayed. This value may range from 40 ms to 30,000 ms. The display is centered about a user selected code in the in search sequence; however, the display may be shifted backward or forward in time so that the centering code is not in the middle of the display window. The program allows the user to mark one event code or set of codes in the search sequence with a small open square on the display. If the user wants to see where other event codes, appear in the raster, these codes can appear as small crosses.

To aid in displaying the data in graphic form, the program allows different display formats to be specified. The display format records may include histograms of specifiable binwidth, reciprocal interval plots (RIPs) and rasters of the pulse data. They also may include traces of the analog signals (e.g., limb position and their derivatives).

There are several statistical calculations which can be made by the program. One is based upon a non-parametric comparison of spike in two specifiable raster segments. Sometimes it is desirable to compare pulse data in different rasters. This may be accomplished by creating specific data files and subsequently performing the test on these files.

Significance to Biomedical Research and to the Program of the Institute

This study continues a fundamental line of experiments aimed at understanding the role that the pyramidal tract neurons and the basal ganglia play in the control of movement. Previous studies have been concerned with which aspects of movement to which neuronal activity in primary somatic sensorimotor cortex is related, whereas these experiments have investigated more closely information processing occurring within cortical columns, the elementary functional unit of cortex. Continuing experiments using awake monkeys, trained to perform voluntary movements, will add to our understanding of how changes in cerebral activity modify behavior in response to afferent information. The studies of the basal ganglia open a new perspective into its mechanisms of function. In particular, the finding that tonically active cells, widely distributed throughout the basal ganglia, can have their activity synchronized in a set-dependent manner, suggests those cells are important in the regulation of normal functioning of the basal ganglia. Further studies of the neurophysiological and neuropharmacological correlates of the tonically active basal ganglia neurons should illuminate mechanisms of basal ganglia dysfunction in neuropsychiatric disorders.

The new neurophysiological analysis program enables the laboratory to extract more information from its data, and to do so more efficiently. The major improvement over previous programs used in the laboratory is that it is interactive. Previously, all analysis had to be performed by a limited batch (non-interactive) program. The new program provides extensive and flexible sorting capabilities not available before. Other features which were not previously available are signal processing, creation of output files for easy access to data for further special analysis, and calculation of instantaneous cell discharge frequency. The program also provides an extensive graphics capability for creating custom tailored, publication quality graphics.

Proposed Course:

Experiments, parallel to those described in this report, have begun with monkeys trained to perform voluntary forelimb movements. The goal of these experiments is to assess how motor cortex responsiveness may change during the preparation and/or execution phases of voluntary movement. Our working hypothesis is that changes in responsiveness of the output elements (PTNs) to cerebellar and/or peripheral inputs will occur during dynamic phases of movement.

Plans for future development of the computer analysis program include writing an enhanced, portable version for more contemporary computers, including the VAX. This will greatly increase the speed and sophistication of interactive neurophysiological analysis.

Publications:

Evarts, E.V.: Emergent features in motor cortex as keys to negative symptoms following pyrimidal tract lesions. In Delwaide, P.J. and Young, R.R. (Eds).: Clinical Neurophysiology in Spasticity. Elsevier Science Publishers, Amsterdam, 1985, pp. 13-25.

Evarts, E.V.: Hierarchies and emergent features in motor control. In Edelman, G.M., Gall, W.E. and Cowan, W.M. (Eds).: Dynamic Aspects of Neocortical Function. Neurosciences Institute, John Wiley and Sons, New York, 1984, pp. 557-579.

Evarts, E.V.: Transcortical reflexes: Their properties and functional significance. In Goodwin, A.W. and Darian-Smith, I. (Eds).: Hand Function and the Neocortex. Springer-Verlag, New York, 1985, pp. 130-154.

Evarts, E.V., Kimura, M., Wurtz, R.H., Hikosaka, O.: Behavioral correlates of activity in basal neurons. Trends in Neurosciences 75: 447-53, 1984.

Kimura, M., Rajkowski, J., Evarts, E.V.: Tonically discharging putamen neurons exhibit set-dependent responses. Proc. Nat. Acad. Sci. (USA), 81: 4998-5001, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01090-09 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Central Nervous System Functional Anatomy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others:	Sandra Moon Edley	Staff Fellow	LNP, NIMH
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	Stafford McLean	Staff Fellow	LNP, NIMH
	Richard B. Rothman	Guest Worker	LP-DSMHR, NIMH
	Candace B. Pert	Biochemist	NSB, NIMH

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TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A sensitive method for light microscopic localization of brain receptors by in vitro autoradiography was developed previously in this laboratory. By this method we have mapped the locations of opiate receptors in the brains of rats and other vertebrates, including primates. Comparisons of radiolabeled opiate alkaloid binding with radiolabeled enkephalin binding have confirmed the existence of opiate receptor subtypes. A similar strategy led to the optimization of binding conditions to selectively reveal the distribution of substance P receptors and eledoisin binding sites. Immunohistochemistry is used to compare the distributions of putative neurotransmitters and their receptors. The relationship of these localization patterns with other markers of brain heterogeneity, provided by tract tracing and enzyme staining, allows hypotheses about functional circuitry in the central nervous system.

Objectives:

Over the last decade a major thrust of neuroscience research is the identification of neurotransmitter, neuromodulator and hormone receptors in the brain. An understanding of receptor function requires knowledge of the biochemistry and pharmacology as well as the neuroanatomical localization of receptors. Meaningful receptors are identified by pharmacological criteria in collaborative studies with Dr. C. B. Pert, Clinical Neuroscience Branch, and Dr. R. B. Rothman, Laboratory of Preclinical Pharmacology, NIMH. We next seek to identify the neuronal circuitry that is "plugged into" these receptors by comparison with known anatomical pathways and by immunohistochemical identification of transmitter-specific connections. Other main objectives are to understand the role of a receptor or receptor subtype in any given region by determining receptor density, maleability in tests of developmental time course or pharmacological manipulation, and altered distribution in neuropathological tissues.

Methods Employed:

We have successfully developed an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue sections. Fresh, frozen cryostat-cut brain sections are securely attached to glass slides by a process of thaw-mounting and subsequent drying at cold temperatures. Slides are then incubated in solutions containing radiolabeled ligands. Excess and non-specifically bound ligand is washed off in cold buffered rinses, and the slides are blown dry. The sections are fixed in hot formaldehyde vapors under a vacuum, defatted in xylene and alcohol rinses, dried and then coated with radioactive-sensitive emulsion for autoradiography. Alternatively, sections can be placed in an x-ray cassette and overlain with LKB tritium-sensitive film. The developed film autoradiographs then can be analyzed by a densitometer for computer-assisted quantification of receptor densities. While emulsion-coated sections provide high resolution analysis through the microscope, films can be computer-analyzed for rapid quantification of receptor densities or for color-coded image enhancement.

Major Findings:

The method we published for in vitro autoradiographic localization also appears in several books on receptor methodologies. A technical note on the advantages of tissue defatting for quantitative autoradiography, co-authored with Dr. L. Sokoloff, Laboratory of Cerebral Metabolism, NIMH, was published. The resolution and signal-to-noise ratios we obtain are better than other workers achieve, so we have been particularly successful at detecting subtle differences in the distributions of receptor subtypes and precise correlations with other anatomical and chemical markers such as cell and fiber stains, immunohistochemistry of transmitters and transmitter-specific enzymes, labeled pathways and catecholamine fluorescence. The technique is also well-suited to study fragile tissues and tissues that require suboptimal binding conditions, and so we have succeeded in studying fetal development and the binding of several "novel" peptides. These findings are outlined in detail below.

Many of our studies of opiate receptor localization in relation to other functional, morphological and developmental markers have focused on the basal gang-

lia, structures subserving both "mood and movement" by a number of criteria. Within the basal ganglia, the neostriatum is richly innervated by dopamine fibers originating from the A8, A9 and A10 midbrain cell groups. The pattern of striatal dopamine fluorescence undergoes marked changes in the perinatal period in rats; we showed this as well as the developmental relationship of dopamine islands and opiate receptor patches. The role that receptors play in influencing the ontogenetic sequelae was studied by giving dopamine and opiate receptor blockers (antagonists) via osmotic minipumps to pregnant rats during the critical prenatal period. In another study, sex differences in opiate receptor distributions in the preoptic area were noted. Both studies showed that the adult patterns could be altered by hormonal manipulations in the early postnatal period. These results have implications for human brain development, especially in situations of maternal drug consumption.

The slide-mounted sections have turned out to be superior to brain homogenates or membrane preparations in several biochemical studies of binding kinetics, both in the opiate and substance P systems. The reasons for this are not known, but problems with nonspecific binding to glass filters are obviated, and it is possible that the more intact nature of the tissue sections facilitates demon stration of events that normally occur in vivo. Further improvements in signal-to-noise ratios may be obtained by using iodinated rather than tritiated ligands, and the higher energy isotopes greatly reduce the exposure times needed for autoradiographic visualization. In the studies of characterization of receptor subtypes after optimizing subtypeselective binding conditions by modifying buffer content, temperature, ions and allosteric effectors, and the addition of protease inhibitors and agents that block (sometimes irreversibly) one or more sites recognized by the ligand, autoradiographs reveal characteristic patterns which identify and validate distinct and discrete subtype distributions in brain. These have important implications for development of drugs for human use that are site-selective and, therefore, have precise actions and fewer side effects.

The recent wealth of publications showing maps of neurochemical markers, by the techniques of autoradiography and immunohistochemistry, has permitted a first careful analysis of the common expectation that the maps of transmitter distribution (at nerve terminals where release occurs) ought to closely resemble maps of associated receptor distributions. A clear violation of that expectation was provided in the substance P system. The work of our group and others showed that the striatonigral projection system in the rat contains substance P. The density of this putative transmitter in terminals within the substantia nigra is the highest in the rat brain. Working with Dr. Richard Rothman (project no. Z01 MH 01584-01 LP), we next optimized in vitro conditions for the binding of [^{125}I]substance P and [^{125}I]eledoisin to two subtypes of the substance P receptor. To our surprise we found that each peptide bound to receptors heterogeneously and uniquely distributed in brain, but neither bound to the substantia nigra. In fact, the distribution patterns of receptors were not correlated at all with the distribution pattern of substance P in the brain. A similar, though less dramatic, picture emerged after comparing the distributions of the opioid peptides -- enkephalin and dynorphin -- with the distributions of the major opiate receptor subtypes -- mu, delta and kappa. For example, the globus pallidus contains the highest concentration of opioid peptides in the brain, but only low amounts of opiate receptors (mostly kappa). Similarly,

the hypothalamus has high peptide concentrations and low receptor densities. By contrast, the thalamus and portions of the cortex have very high concentrations of all three receptor subtypes (in characteristic patterns) but very low levels of opioid peptide immunoreactivity. These mismatches are pervasive throughout all well-characterized and mapped neurotransmitter/receptor systems, and since many cannot easily be explained by claims of technical failure, such as antibody recognition problems or inadequate characterization of receptor subtypes, we have emphasized several more innovative explanations. These include notions of low-affinity synaptic receptors, high-affinity nonsynaptic receptors, transmitter excesses in storage pools, and diffusion of transmitter to distant targets. These ideas warrent further experimental testing. The concept of "parasynaptic" communication, put forth by others as well as ourselves, has implications for our understanding of drug actions on brain function, as exogeneously administered drugs may mimic normal physiology more than previously considered.

Significance to Biomedical Research and to the Program of the Institute:

The visualization by autoradiographic techniques of opiate receptor locations throughout the CNS ahs greatly advanced our appreciation of the richness of opiate functions in normal physiology and has led to a surprising number of insights into receptor-mediated brain processes. We have just begun to appreciate how receptors influence and control neuronal development and the establishment of neural connections, the interrelatedness of receptor subtypes and neurochemical systems, the evolution of receptors as markers of synaptic and nonsynaptic complexity and the significance of species differences. When these systems and processes are understood in detail, significant improvements can be expected in the approach to neuropsychiatric disease.

Proposed Course of the Project:

The findings obtained this year indicate a productive future for the present approach in research into brain function. Using combinations of receptor binding, immunohistochemical, pharmacological and physiological techniques, we will be examining the distributions of receptors in several neurotransmitter systems with careful attention to their potential as mediators of classical as well as non-classical forms of cell-to-cell information transfer. Application of these techniques to the study of alterations in the brains of humans with histories of mental disorder is being evaluated.

Publications:

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Herkenham, M.: Autoradiographic methods for receptor localization. In Karten, H.J. (Organizer): Modern Neuroanatomical Methods. Neuroscience Short Course #1 Syllabus. Soc. Neurosci., Bethesda, Maryland, 1985, in press.

Herkenham, M.: Levels of quantitative analysis of receptor autoradiography: technical and theoretical issues. NIDA Research Monograph, in press, 1985.

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Moon, Sandra L.: Prenatal haloperidol alters striatal dopamine and opiate receptors. Brain Res. 323: 109-113.

Moon Edley, S. and Herkenham, M.: Comparative development of striatal opiate receptors and dopamine revealed by autoradiography and histofluorescence. Brain Res. 305: 27-42, 1984.

Rothman, R.B., Herkenham, M, Pert, C.B., Liang, T., and Cascieri, M.A.: Visualization of rat brain receptors for the neuropeptide, substance P. Brain Res. 309: 47-54, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01091-08 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Motor Function in Patients with Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jerome N. Sanes	Senior Staff Fellow	LNP, NIMH
Others:	Edward V. Evarts	Chief	LNP, NIMH
	Mark Hallett	Clinical Director	NINCDS

COOPERATING UNITS (if any)

Section on Human Motor Control, Medical Neurology Branch, NINCDS

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purposes of this project are to examine the contributions of central motor programming and afferent input in control of arm movements in normal subjects and patients with sensorimotor disorders, and to study psychomotor performance of patients with central motor disorders. The first set of experiments records muscle activity and kinematics of limb position while (1) subjects manually match a target display with either a skilled rapid or slow movement with a handle whose displacement controls a visual display or (2) maintain postures when limb position is passively changed. Movement amplitude, presence or absence of visual feedback or position, disturbance of the subject's movements and changes in sensory input are independent variables. Large movements are performed accurately independent of manipulation of the experimental variables but accurate performance of small movements becomes increasingly dependent on the absence of limb disturbances during movement. The second set of studies examined a variety of psychomotor variables from patients with a variety of neurological disorders. Voluntary and involuntary movements are evaluated to develop sensitive measures of psychomotor performance.

Objectives:

The importance of afferent information in the control of limb movements is controversial. Whereas it is clear that afferents exert potent physiological effects on spinal motoneurons and cells in supraspinal structures, it has been suggested that some of these afferents contribute little to the final positioning of a limb. A case in point is the observation that muscle spindle activity does not reflect muscle length during rapid movements of large amplitude, thereby casting doubt on a regulatory role for spindles at the end of movements. In addition, physical disturbances imposed during movements, that likely activate muscle spindles, do not appear to modify final limb positioning. There are, however, other experiments demonstrating the importance of afferent input in a variety of tasks performed by humans. For example, ischemic deafferentation of limbs alters position sense and sense of effort. Furthermore, performance of fine motor tasks, such as reproduction of alphabetic characters is also disrupted by ischemic deafferentation and it is noteworthy that inactivation of the gamma loop in humans impaired the ability to tonically activate motor units, though phasic activation was not impaired. It is the object of the present project to continue examination of the role of peripheral inputs in the control of limb movements and postural control. In addition, the organization of central processes of movement will be explored further. In many of these experiments the trajectories and end-points of two-dimensional movements about the elbow and shoulder will be evaluated. Both normal volunteers and patients with neurological disorders will be studied during performance of movements of varying sizes and when a maintained posture is disturbed by different peripheral inputs. Different types of limb disturbances will be imposed during the movements. Two general experimental approaches are being pursued. In the first, the psychomotor variables of movement error and movement time are studies in relation to physical disturbances. In the second group of experiments, electromyographic activity is examined when the limb is mechanically perturbed while subjects perform motor tasks or maintain postures.

Methods Employed:

Human subjects are trained to manipulate a handle that is attached to a servo-controlled torque motor while performing extension-flexion of the wrist, elbow or index finger, or abduction-adduction of the index finger. Displacement of the handle causes movement of an oscilloscope beam that is to be matched by the subject with a second, experimenter controlled, oscilloscope beam. In one series of experiments, subjects perform tracking movements either as rapidly as possible or as accurately as possible. For a variety of movement sizes (3° to 30°) subjects are given an adequate number of training trials. Independent variables include (1) continuous loads opposing or assisting movement, (2) brief physical disturbances delivered to the arm before or after initiation of arm movement and (3) initial starting position and (4) full or partial information concerning the direction and extent of the instructed movement. Patterns of muscle activity and tracking errors are analyzed during rapid movements.

Main Findings:

Motor behaviors during movements of normal subject and patients with large fiber sensory neuropathy or cerebellar disease have been studies. Several findings have emerged:

(1) The sense of effort was evaluated in normal subjects and in patients with a large-fiber sensory peripheral neuropathy. Two unimanual and one bimanual detection tasks were used. In the first unimanual task patients were required to maintain a constant posture of 0° wrist rotation while torques were changed every 4-5 seconds to one of six levels opposing flexion (0-0.8 Nm). In the second task, the right hand of patients was pushed against a mechanical stop (45° extension) by a torque opposing flexion and were required to move to 0° of flexion. In both of these tasks the patients had to discriminate whether the torque had changed in comparison to the torque of the previous trial. Patients responded, "more", "less", or "same" on all trials, and the proportion of correct responses was tabulated for trials when torque actually changed and for trials when there was no change in torque. A bimanual matching task was also used in which a constant torque opposing flexion (0.16-0.8 Nm) was continuously applied to the left hand and a varying torque opposed flexion of the right hand. Subjects were required to judge onto which hand had the larger applied torque. A modified Method of Limits procedure was used to determine ascending and descending thresholds. With this procedure torque on the matching right hand started at 0 Nm and was raised in 0.5 Nm steps until the patient made four successive responses that torque was greater on the matching arm. Then torque was lowered in 0.5 Nm steps until four responses of torque greater on the reference arm occurred. Increases and decreases in torque were done for 5-10 approximations of the ascending and descending thresholds.

The results from these experiments showed that patients with a large-fiber peripheral neuropathy have a severely impaired sense of muscular effort. This was apparent in both the unimanual and bimanual tasks. Closer inspection of the data revealed that patients do have a crude sense of effort. In particular, in the unimanual tasks, most errors that patients and normal controls made were when the change in torque was less than 0.5 Nm, but of course patients made more of these errors.

(2) In previous annual reports we had described some mechanical physiological and cognitive variables that affected intention tremor presented by patients with cerebellar disease. This type of tremor was influenced by the presence or absence of visual guidance: tremor was reduced when any segment of the visual guidance feedback loop was opened. Mechanical factors also altered cerebellar tremor. Viscous and inertial loads diminished the tremor, with very small viscosities and inertial loads providing a disproportionate amount of tremor reduction. Loads that activated extensor muscle groups exacerbated tremor while tremor was reduced by activation of flexor muscles. New work in these investigations has focused on the patterns of muscle activity during mechanical conditions that diminish tremor and force recording during isometric muscle contractions. Analysis of EMG records showed that tremor in cerebellar patients is typically accompanied by alternating bursts in antagonist muscles about a joint. (This contrasts with some other pathological tremors in which the EMG bursts in

antagonist muscle are synchronous but unbalanced). The diminution in tremor of cerebellar patients related to inertial or viscous loads appears to be related to a phase shifting of the antagonist EMGs. Instead of a pure alternating pattern (as seen under normal mechanical conditions), a somewhat overlapping pattern of muscle activities was observed. Thus, the forces exerted by the antagonist muscles tend to cancel each other. This temporal shifting of muscle activities suggest a control by somesthetic afferent inputs of tremor exhibited by cerebellar patients. One apprach to further investigate this issue has been to examine force tremor while cerebellar patients perform isometric muscle contractions. In this situation muscle spindle inputs unrelated to voluntary motor commands are effectively removed from affecting muscular activity. We found that under isometric conditions cerebellar patients do not exhibit intention force tremor. Currently it is unclear whether the reduction of cerebellar tremor during isometric conditions is related to phase shifting of EMG activities in antagonist muscles or overall reduction of EMG activities.

(3) In another investigation of the sensory mechanisms controlling tremor, we investigated whether postural tremor was sensitive to variations in static limb position. Patients with cerebellar ataxia, but not intention tremor, were examined. They were required to position the arm at various angles of forward flexion with or without additional elbow flexion. Although postural tremor was observed at most of the required positions, tremor was greatest when elbow flexion was progressively increased. As with the observations with cerebellar intention tremor, it appears that postural tremor depends upon a constellation of afferent and efferent signals.

(4) In previous annual reports, we described preliminary findings on the role of proprioceptive information in movement control of normal humans and patients with sensory disturbances. The results demonstrated the importance of information derived from large fiber somesthetic afferents for postural stability, force maintenance, performance of discrete movements, especially those of small magnitude, and in the development of enhanced physiological tremor. The importance of somesthetic inputs was particularly apparent in the ability to maintain constant motor output as shown by the failure of "deafferented" patients to sustain a given level of muscle activity or maintain postural stability. The postural instability of deafferent patients was evident, though diminished, even when visual guidance was available. But upon removal of visual guidnce of hand position, patients had no sensory feedback for guidance and any postural drift remained uncorrected. The deterioration of postural maintenance of deafferented patients was typically observed soon after (< 2 seconds) the visual guidance had been removed. It was as if the brain structures controlling levels of excitation to the muscles "forgot" the appropriate level of motor neuronal excitation upon elimination of visual guidance, with the result that motor commands began drifting when visual guidance was eliminated. This rapid deterioration of steady-state output levels suggests that motor memory requires updating, resumably by kinesthetic afferents, of the consequences of intended motor output. In addition to deficits in posture, phasic motor control was also impaired in the patients with sensory loss since discrete movements were poorly performed. Without vision, the patients would typically overshoot or undershoot the target and then briefly maintain the new position. Thus, it appears that the termination of discrete movements also depends on somesthetic afferent input.

The studies with normal subjects that were concerned with the role of proprioceptive information in movement control indicated that unexpected mechanical perturbations occurring at the beginning of movement disrupted accuracy of small movements more so than the accuracy of large movements. These behavioral results occurred despite the observation that larger "compensatory" muscle responses were triggered by the perturbations occurring during large movements. It might be related to accuracy but instead the relationship between the voluntary and triggered muscle responses are critical in determining whether an accurate end-point position will be achieved. Since small movements are caused by small amounts of muscle activity compared to the electromyogram seen with a maximum contraction it could be stated that a relatively small proportion of the motor neuron pool is activated by a motor command signaling a small movement. In contrast, large movements are caused by relatively large amounts of muscle activity compared to that observed with a maximal voluntary contraction and a relatively large portion of the motor neuron pool is recruited by a motor command signaling a large movement. Thus, small movements probably recruit only low threshold motor units while large movements recruit low and medium threshold motor units. An additional observation on the organization of the motor neuron pool suggests why small movements are more affected by perturbations. That is, the motor units in any pool with relatively low thresholds are obviously more easily recruited and probably easier to modulate in discharge rate than high threshold motor units. If only low threshold motor units are recruited for small movements excitatory peripheral inputs would be more likely to recruit motor units with slightly higher thresholds. In contrast, peripheral inputs occurring during large movements would have to raise the excitability level of the motor neuron pool more to recruit new motor units than if small movements were performed. Therefore, the summation of the motor command and the triggered EMG reaction would appear to be the important variable in determining whether movements were accurate or inaccurate.

Significance to Biomedical Research and to the Program of the Institute:

Additional clarification of how somatosensory information and central motor commands are used to control skilled motor activity is essential to the understanding of normal and abnormal motor behavior in humans.

Furthermore, these studies will provide standards of normal motor function and allow comparisons with patients wih motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents. The objective evaluation of neuropsychiatric disorders that we have developed should prove useful in a wide variety of experimental applications that require computer recording and analyses of results. Long-term evaluation of patients' progress on medication regimens in particularly suited for objective analysis.

Proposed Course of the Project:

This research project will be discontinued in the LNP. However the work will continue in NINCDS with collaboration from members of the LNP.

Publications:

Sanes, J.N. and Evarts, E.V.: Psychomotor performance in Parkinson's disease. In Delwaide, P.J. and Agnoli, A. (Eds): Clinical Neurophysiology in Parkinsonism. Elsevier Science Publishers B. V., Amsterdam, 1985, pp. 117-132.

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Sanes, J.N.: Absence of enhanced physiological tremor in patients without muscle or cutaneous afferents. Journal of Neurology, Neurosurgery & Psychiatry, in press.

Sanes, J.N., Mauritz, K.-H., Dalakas, M. and Evarts, E.V.: Motor control in humans with large-fiber sensory neuropathy. Human Neurobiology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01092-07 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Nonprimary Motor Cortex and the Cerebral Control of Movement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Steven P. Wise	Research Biologist	LNP, NIMH
Others:	Kiyoshi Kurata	Visiting Fellow	LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.7	1.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three of the main cortical inputs to the primary motor cortex (MI) are the premotor cortex (PM), the supplementary motor cortex (MII) and the transition zone between the motor and somatic sensory cortex, area 3a. These three cortical fields surround MI and, in this project, were differentiated from MI on the basis of neuronal responses to peripheral inputs, thresholds for evoking movements with intracortical electrical stimulation, the properties of single neurons during the performance of an operantly conditioned motor task, cytoarchitecture, and connectivity. In our most recent work, we have concentrated on one of these fields, PM, and an analysis of its neuronal activity during a variety of visually guided motor tasks. Each task was designed to elucidate the role of PM in the cerebral control of movement. We have tested the following hypotheses: (1) that PM guides movement to points in space, (2) that PM is involved in the sensory guidance of movement, (3) that it is especially important when such sensory guidance is of an abstract nature, (4) that PM plays a role in motor preparation, (5) that it functions in the determination of movement parameters, (6) that PM is especially important in controlling sequences of movement, and (7) that it reflects eye position, gaze position, postural muscle activity, visual fixation, attention, motivation, or arousal. Of these ideas, our results support the hypothesis that PM plays a role in the execution of visually guided movements, especially those guided by astract sensory cues, and the preparation for voluntary movements. These studies have provided new insight into the process termed preparatory set, which may underlie the ability of animals to make advantageous preparations for actions in the future. As such, it represents a higher brain function amenable to both quantitative and qualitative neurophysiological analysis.

Objectives:

The inputs to the precentral motor cortex (MI) and its intrinsic neuronal circuitry determine the output of MI neurons, including those projecting to the spinal cord. It has only recently been fully recognized that the number of motor cortical fields supplying inputs to MI includes a premotor cortex (PM) as well as the long-known supplementary motor cortex (MII). Thus, the motor cortex includes in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery. The goal of this project is to gain a better understanding of the cortical fields supplying inputs to the MI cortex and their interaction in producing motor output. A particular focus is on those cortical fields involved in controlling movements guided by sensory inputs, such as vision. Our overall objective is to come to a better understanding of the processing underlying the behavioral flexibility of higher mammals - i.e., the ability to respond with virtually any motor action of which the animal is capable to virtually any sensory input that the animal can discriminate.

Methods Employed:

Eight rhesus monkeys have been trained to perform visually guided motor tasks. Each one of the six tasks described below is considered to be a separate experiment. (1) One monkey was operantly conditioned to depress one of four keys located in a perimeter at arms length. While the monkey pressed one key, another of the four keys, selected randomly, was illuminated after a randomly varied delay. This key thereby became the next target. A barely discernable visual cue near the target key, appearing after another variable delay, signaled the monkey to move and depress the target. The monkey was required to make the movement within a short period of time, near the limit of reaction time. Neurons in PM were studied in this experiment. (2) Two monkeys were conditioned to align two spots of light on a tangent screen in front of the monkey. One of these spots is controlled by a computer (the target spot), the other by the arm movements of the animal (the position spot). The monkey was required to align the spots within a small accuracy "window." In five-sixth of the trials, after a short period of time the target spot jumped to one of six locations. The monkey had to maintain his arm position unchanged until the target spot dimmed, at which point he was required to flex or extend his forearm rapidly and accurately in order to realign the position spot with the target spot. In one-sixth of the trials, the computer selected a situation in which physically identical stimuli signaled the animal to make no movement. This experiment was designed for two purposes: to contrast neuronal activity in MI and PM, and to distinguish neuronal activity when identical stimuli signal the execution vs. the withholding of movement. (3) Two monkeys were conditioned to depress the central key of three keys located on a panel in front of the animals. After a period of time, either the left or right key became illuminated. Three experimental conditions ensued: (a) the left or right key remained illuminated and served as the target for the subsequently triggered movement, (b) the light was turned off before the monkey was allowed to execute the movement, forcing the monkey to remember the proper target, or (c) the target light was switched before the monkey was allowed to execute the movement. This experiment was designed to further test the relationship of neurons in PM to the preparatory set of the animal.

Of special interest were the conditions in which the signals were absent or the preparatory set changed during the course of a trial. (4) One monkey was conditioned

to execute a single limb movement as well as a short sequence of two limb movements in the same direction. The monkey was seated in front of a panel of three keys as in experiment #3. Each trial started with the monkey pressing the leftmost of the three keys. Two experimental conditions ensued: (a) the center key was illuminated, thus indicating that a single movement was to be made to depress the center key, or (b) both the center and right lights were simultaneously illuminated to indicate that a short motor sequence was to be initiated to depress both keys, in order. This experiment was designed to test the hypothesis that PM is especially important in guiding sequences of movement. (5) One monkey was conditioned to respond to two different sorts of visuospatial instruction signals. One type of instruction signal was comparable to that described in the foregoing experiments, i.e., the visual cue itself directly indicated the direction that the limb was to be moved upon receipt of a subsequent triggering cue. These movements were termed "directly" guided movements. The simplest example of a directly guided movement, as in experiments #1 and #3, is when the instruction light was part of a target key to be hit. This situation could be contrasted with one in which the instruction cues contained no directional information, i.e. a blue lamp meant to move the limb to the right and a yellow lamp to move to the left. These movements were termed "abstractly" guided movements. Thus, premotor cortex activity could be compared in response to "direct" and "abstract" visual cues. (6) One monkey was conditioned to respond to cues under two different experimental conditions: (a) "direct" visual instructions of the type described for experiment #5 guided the movement, and (b) the identical cues were irrelevant and the monkey guided its behavior via internal (i.e. nonsensory) processes. This experiment was designed to test the hypothesis that PM is especially important when sensory signals guide a movement and to contrast activity in PM with that in MII, hypothesized to play a special role when internal processes guide a movement (see Evarts and Wise, 1984; Wise and Mauritz, 1985).

Single-unit activity and behavioral data were collected on-line with a PDP 11/03 computer and analyzed off-line with PDP 11/34 and PDP 11/23 computers. Presently, we are collecting and analyzing the data with a dedicated PDP 11/23 in the laboratory, using an analysis program written by Karl F. Arrington of the LNP support staff.

Following the recording procedures, small amounts of a biological tracer may be injected into either PM, MI or MII. By noting the ultimate distribution of the tracer in the brain, the sites of termination of neurons in the somatic sensorimotor cortex or inputs to these cortical fields can be determined by histological techniques.

Major Findings:

About 1650 units have been examined in this project to date, and 795 of these have been studied in detail. Two major sets of findings developed from our earlier work on this project.

1. MII and PM neurons were found to be much less responsive to peripheral somatosensory inputs than MI neurons in the same monkeys. The lack of profound somatic sensory responsiveness in these parts of the somatic sensorimotor cortex supports the hypothesis that MII and PM play their most significant roles in the guidance of movement by exteroceptive stimuli (such as vision and audition) or internal processes (such as memory) rather than by feedback from mechanoreceptors of the limbs. MI seems to be specialized for control of movement, in part, by cutaneous and noncutaneous mechanoreceptors, and this line of investigation is being developed by Von Jennings in a related project.

2. Our findings have enabled us to improve the current understanding of cerebral localization in the agranular frontal cortex and certain adjacent parts of the cortex regions. Of special importance has been the effort to determine anatomical correlates of physiologically defined cortical regions. Microelectrode methods revealed that the boundary between MI and MII corresponded to the boundary between two anatomically defined parts of the agranular frontal cortex (termed areas 4 and 6). This differed from the accepted published maps at the time this study was undertaken, and this line of investigation has been taken up again in the laboratory in a new, related project headed by Andrew Mitz. Similar work has clarified the location of the boundary between PM and MI.

The findings described above led to the elaboration of a related, third set of findings, those concerning the premotor cortex. The premotor cortex can be distinguished from the MI representation by its markedly increased threshold for evoking movements with intracortical microstimulation. Further, and of most interest to us, a substantial population of neurons change their activity in relation to preparatory set and/or the signals that indicate the location of movement targets. One class of cell in premotor cortex, termed "set-related neurons," appear to be specifically correlated with the motor preparation (or preparatory set) of the animal. This hypothesis has been supported in three ways: (a) set-related units show changes in activity when visual signals cue a movement, thus establishing a specific preparatory set, but not when the same signals instruct the monkey to withhold movement, (b) if the visual instruction changes (to establish a different preparatory set) before the movement, the unit activity rapidly changes to reflect the new set, and (c) when the instruction is removed (but the preparatory set remains the same), the unit activity continues to reflect the set rather than the sensory signals. In addition, it has been found that these and other premotor cortex units change their activity in relation to predictable environmental events, and that activity in the premotor cortex is comparable before a single movement and a fixed sequence of two movements in the same direction. Our most recent results indicate that set-related activity before movements instructed by <u>direct</u> cues (see Methods, experiment #5) is greater than or equal to that activity before <u>abstract</u> visual cues. The latter finding improves our understanding of the set-related processes being performed in the premotor cortex and accord with the hypothesis that PM plays an important role in movements guided by abstract sensory cues.

<u>Significance to Biomedical Research and to the Program of the Institute:</u>

Studies of functional localization in higher-order motor cortical fields, such as the premotor cortex and supplementary motor cortex, are important to under-

standing the cortical control of motor acts of the least automatic kind, in both health and disease, and especially for understanding the way in which sensory signals are converted, by the brain, into organized motor acts. A much improved knowledge of the nonprimary areas of the cerebral cortex and their relation to higher-order control of motor behavior may yield insight higher brain functions of all types.

Proposed Course of the Project:

The past year has been devoted to conducting experiments #4 and #5 (see Methods), writing two full-length reports of the results of experiment #3, and writing a third full-length paper comprising data from experiments #1, #2, and #3. The first of these three papers has now been published in the Proceedings of the Royal Society of London, the second has been accepted for publication in Experimental Brain Research, and the third is to be published in Progress in Brain Research as part of the proceedings of a symposium held in Düsseldorf, West Germany last fall. Further, a review of recent work on the premotor cortex was prepared for publication in a special issue of Behavioural Brain Research, based on a meeting on the premotor cortex in the Netherlands. In addition, animals were trained for experiment #6.

Included in the list of publications, below, are the papers prepared by Dr. John Donoghue in the terminal phase of project Z01 MH 01094-04 LNP, which focused on the somatic sensorimotor cortex of rodents. Our work on the physiological organization of the rodent motor cortex has now been brought to a conclusion.

Our proposed course is to further examine the functional organization of motor cortex by acquiring more knowledge about the differential roles of the various fields within the somatic sensorimotor cortex, especially those involved in higher-order control of movement. Most of our recent work has been directed toward the premotor cortex, but we intend to devote more effort in the near future to another field involved in higher-order motor control, the supplementary motor cortex (as outlined in experiment #6 of the Methods). Thus, this project will be continued in the Laboratory of Neurophysiology. We expect the continuing collaboration of Dr. Kiyoshi Kurata, a Visiting Fellow of the Fogarty Foundation, who has special expertise in studying the supplementary motor cortex.

Publications:

Donoghue, J.P.: Contrasting properties of neurons in two parts of the primary motor cortex of the awake rat. Brain Res. 333: 173-177, 1985.

Donoghue, J.P., Wenthold, R.J. and Altschuler, R.A.: Localization of glutaminase-like and aspartate aminotransferase-like immunoreactivity in neurons of the cerebral cortex. J. Neuroscience, in press, 1985.

Evarts, E.V. and Wise, S.P.: Basal ganglia outputs and motor control. CIBA Foundation symposium 107, Pittman Press, pp. 83-96, 1984.

Wise, S.P.: Non-primary motor cortex and its role in the cerebral control of movement. In Edelman, G., Gall, W.E. and Cowan, W.M. (Eds): Dynamic Aspects of Neocortical Function, Neurosciences Institute, John Wiley and Sons, New York, pp. 525-555, 1984.

Wise, S.P.: The premotor cortex: Fifty years after Fulton. Behavioural Brain Res., in press, 1985.

Wise, S.P.: The primate premotor cortex: past, present and preparatory. Annual Reviews of Neuroscience 8: 1-19, 1985.

Wise, S.P. and Donoghue, J.P.: The rodent motor cortex. In Peters, A. and Jones, E.G. (Eds.) Cerebral Cortex. Plenum, New York, in press, 1985.

Wise, S.P. and Mauritz, K.-H: Set-related neuronal activity in the premotor cortex of rhesus monkeys: Effects of changes in motor set. Proc. Roy. Soc. (Lond.), Series B, 223: 331-354, 1985.

Wise, S.P. and Strick, P.L.: Physiological and anatomical organization of the non-primary motor cortex. In: special issue on frontal lobe function. Trends in Neuroscience 7: 442-446, 1984.

Wise, S.P., Weinrich, M., and Mauritz, K.-H.: Is the premotor cortex worthy of the name? Progress in Brain Research, in press, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01093-07 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Somatic Sensory Inputs in the Cerebral Control of Movements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Von Jennings	Staff Fellow	LNP, NIMH
Others:	Steven P. Wise	Research Biologist	LNP, NIMH

COOPERATING UNITS (if any)

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Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines how somatosensory inputs to the primary motor cortex (MI) and somatosensory cortex (SI) influence motor behavior in primates. In particular, the hypothesis that MI neurons respond to mismatches between actual and intended movements was examined by stopping wrist movements in monkeys seeking to make accurate movements. It was found that the response of MI neurons to stopping a movement depends on the distance between the stop position and intended terminal position as well as the joint angle at which the stop occurred. In addition, during unstopped movements some MI neurons responded to the degree to which a movement undershot or overshot the intended target. It is concluded that MI activity reflects the magnitude of the deviation from an intended displacement. This finding provides an important clue concerning the role of peripheral inputs to motor cortex in the initiation and control of movement.

Objectives:

MI neurons receive somatosensory inputs from a variety of receptors in joints, muscles and skin. In addition, some neurons in MI are known to project directly to alpha motoneurons. However, the significance of this transcortical reflex loop remains unclear. One possible role may be to contribute to muscle responses that compensate for a mismatch between actual and intended movement. While it has been shown that the sign of the MI response to a perturbation is appropriate to produce a compensatory muscle response, the quantitative relation between magnitude of cortical responses and extent of mismatch has not been systematically studied.

Another question of considerable importance concerns the route by which somatosensory information reaches MI neurons. Previous experiments in this project addressed this question by studying the activity of SI neurons in regions that are known to be densely and reciprocally connected to MI. Neurons in rostral regions of MI and neurons in posterior SI were found to be strikingly similar in their relation to actively held limb posture and exerted force. Thus, it is possible that some peripheral inputs to MI are relayed through SI. Further evidence for this possibility is being sought by comparing the pattern and timing of MI and SI responses to perturbations during active movement. This information will be of significant theoretical importance concerning the role of sensory feedback to the sensorimotor cortex in the initiation and control of voluntary movement.

Methods Employed:

Monkeys, with their hands attached to a handle, were operantly conditioned to perform wrist flexion-extension movements between two hold zones ("start" and "target") of 1.5° separated by either 7.5° ("small movement") or 15° ("large movement"). The hold zones were shifted together over a 15° range so that constant amplitude movements were made in different angular regions of wrist arc. During randomly chosen trials, movements were stopped at a variable angular position (the "stop position") for 200 ms by a servo-controlled torque motor. Three task variations (A, B, C) were used. In task A, the distance between the stop position and target position (the "remaining distance") was either held constant (at 3°) for both large and small movements or varied (4.5° for small, 12° for large). In task B, only large movements were performed and the stop position was held constant while the remaining distance was varied. In task C, the remaining distance was kept constant (at 12°) by shifting the stop position along with the start and target positions. Three effects of stop were examined: (1) the change in muscle (EMG) activity, (2) the change in single unit activity and (3) a sustained build-up of torque applied by the monkey against the handle. These stop effects were analyzed in relation to the (1) movement size (task A), (2) magnitude of the remaining distance (task B) and (3) stop position (task C).

Activity from single MI and SI neurons and codes specifying certain behavioral events such as movement onset and stimulus presentation were recorded on-line with a PDP-11/03 computer. Analog signals of handle position, velocity and torque and muscle activity from wrist flexor and extensor muscles were recorded

on magnetic tape. Off-line analysis of single unit activity with a PDP-11/23 computer involved a comparison of the pattern and frequency of neuronal discharge during stopped and unstopped movements. A Data Precision 6000 signal averager was used to compare the rectified and filtered muscle activity during these same movements.

Major Findings:

Effects of Stop on Muscle Activity and Torque Production

1. Stops caused increases in agonist and decreases in antagonist EMG activity at latencies of 15 ms to 45 ms.

2. In task A, stops of large and small movements with the same remaining distance produced stop responses and torque build-up with similar magnitudes. In contrast, when the remaining distance was greater for the large movement than the small movement, the magnitude of the stop response and torque build-up was also greater for the large movement.

3. In task B, both the magnitude of the stop response and torque build-up were proportional to the magnitude of the remaining distance.

4. In task C, the magnitude of the stop response increased as the stop position was shifted towards joint angles at which the muscle was shorter. In contrast, the torque build-up was independent of stop position.

Effects of Stop on Single Unit Activity

A. Primary Motor Cortex (MI)

1. Of 481 neurons studied in MI of two monkeys, 133 neurons over a wide area of the forelimb region of MI responded to stopping movements at a latency of 20 ms to 65 ms. Neurons that increased their activity early (prior to movement onset) tended to have heightened increases in activity following a stop. In contrast, neurons showing increases in activity at or after movement onset tended to be inhibited when displacements were stopped.

2. The stop response of many MI neurons resembled the stop response of prime mover muscles. These neurons tended to be located in rostral regions of MI and usually showed phasic bursts of activity that began before movement onset in one (the "preferred") direction. In general, neurons with the largest stop responses also had the largest premovement phasic burst of activity.

3. In task A, these neurons showed a larger stop response during stops of large than during stops of small movements when the remaining distance was greater for the large movements. In contrast, stops of large and small movements caused similar stop responses when the remaining distance was the same.

4. In task B, these neurons showed a graded stop response that was proportional to the magnitude of the remaining distance. The stop response increased by approximately 11 IPS/deg as the remaining distance increased from 1.5° to 13.5°.

5. In task C, when the stop position was shifted in the preferred movement direction with the remaining distance held constant, the stop response increased by about 3 IPS/deg.

6. During unstopped movements that initially did not achieve the desired target, some neurons in rostral MI showed an additional burst of activity that began while the limb was still moving toward the undershot position. These same neurons had decreased activity during late stages of movements that overshot the target. This pattern of activity suggests that these neurons were responding to a signal of mismatch between an intended and actual trajectory.

7. Many neurons in caudal regions of MI showed stop responses that were distinctly different from the stop responses of neurons in more rostral MI regions. These caudal neurons tended to become active at or after movement onset and showed decreased activity following a stop. In addition, the stop response of these cells was not affected by changes in either the remaining distance or the stop position. This result is similar to findings of a previous experiment in this project that showed that neurons with cutaneous peripheral inputs (1) are concentrated in caudal regions of MI (MI/c), (2) become active relatively late during movement, and (3) show little sensitivity to actively maintained position or torque.

B. Somatosensory Cortex (SI)

Analysis of SI responses to stopping movements in progress. Thus far, several similarities and differences between MI and SI stop responses have been observed.

1. SI neurons with stop responses similar to those of rostral MI neurons and prime mover muscles were found predominantly in area 2 and area 3a. SI neurons with stop responses similar to caudal neurons were concentrated in area 3b and area 1. While no large differences between the response latencies of MI and SI neurons have been observed, a more quantitative analysis is in progress to determine if a small but significant difference exists.

2. Some area 2 neurons displayed stop responses that were different from any MI stop response. Some of these neurons showed little activity during unstopped movements but displayed large magnitude responses to stopping movements. Other area 2 neurons were very active during movements in the preferred direction but responded to stops of movements in the opposite direction.

Significance to Biomedical Research and the Program of the Institute:

In order to better understand brain mechanisms that generate normal limb movements in man and how these mechanisms are affected by pathological conditions, more information is needed concerning how inputs from the periphery are used to initiate and control skilled motor activity. While it is known that the motor cortex receives somatosensory inputs prior to and during limb displacements, much about the function of these inputs remains to be clarified. An examination of this question will contribute to our understanding of the role of sensory feedback in the cerebral control of movement.

Proposed Course of Project:

While the present experiment involved stopping a movement to show that MI activity reflects the magnitude of mismatch between an actual and intended movement, more information is needed about how MI responds to other causes of mismatch. The next step in this project will be to compare the response of MI neurons to mismatch produced by other external sources, such as changes in applied torque, with the response to mismatch caused either by internal factors or stopping a movement.

Publications:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01095-01 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuroanatomical Interrelations of Chemically Defined Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Charles R. Gerfen Senior Staff Fellow LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A general three-step method was developed to determine the interrelations among biochemically defined neural circuits in the brain: (1) The detailed morphological features of any set of neurons is visualized at the light microscopic level by immunohistochemical localization of the plant lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) which, after injection into the brain, reveals the morphology of labeled neurons perikarya, dendrites and full axonal projection systems. (2) Concurrent localization of biochemical markers and PHA-L provides a means of tracing chemically defined projections. And (3) the projections of neurons that are targets of afferents characterized by step 1 and 2 are determined with retrograde axonal transport of fluorescent dyes. The relationship of such characterized neural systems to autoradiographically labeled receptor systems provides further information regarding the biochemical nature of neural circuits. Studies using this method has provided new insight into the organization of the the basal ganglia. The biochemical compartmentalization of the striatum is shown to reflect the existence of distinct input-output systems which connect the cerebral cortex through the striatum to substantia nigra. Calcium binding proteins are also selectively expressed in specific striatal compartments. Current studies are directed toward the functional significance of compartmentalized input-output organization in the basal ganglia.

Objectives:

In addition to their classically recognized role in motor behavior, the basal ganglia are now thought to function in virtually all aspects of motivated behavior. Alleviation of some symptoms of mental disorders with pharmacological manipulations that affect the basal ganglia demonstrate that motor and affective functions within the basal ganglia are complexly interrelated. One line of reasoning has suggested that the connections of the dorsal and ventral striatum are devoted separately to motor and affective function, respectively. The present projects is directed, in part,to a test of that hypothesis. Concurrently, evidence has accumulated that there are local heterogeneities in the biochemical makeup of striatal regions, and these heterogeneities are related to the neuroanatomical connections of the striatum. The aim of the present project is an understanding of the manner by which separate functional inputs to the striatum may be selectively influenced by neurochemicals. Such understanding is necessary for the development of specific pharmacological approaches to neuropsychiatric disease.

Methods Employed:

1. Combined neuroanatomical methods were used to study the organization of the basal ganglia. The principal method common to most studies involved the anterograde axonal transport method using the plant lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) as an axonal tracer (Gerfen and Sawchenko, 1984). Stereotaxically positioned iontophoretic injections of PHA-L were made into either the cerebral cortex, striatum, or substantia nigra of rats. Following a sufficient survival time to allow for slow axonal transport of PHA-L, animals were perfused with fixative, their brains removed and saturated with sucrose to allow frozen microtome sectioning. 30 um-thick sections were incubated in rabbit antiserum directed against PHA-L and then processed for either lightor fluorescent-microscopic immunohistochemical visualization of injected and axonally transported PHA-L. Retrograde transport of fluorescent dyes injected into the substantia nigra labeled striatonigral projection neurons and, when used in conjungution with PHA-L labeling of corticostriatal inputs and immunohistochemical labeling of striatal compartments, allowed direct examination of striatal input-output systems relative to those compartments. Dual use of PHA-L and autoradiographic tract-tracing methods showed the relationship of striatonigral projections from different striatal regions.

2. In another study, PHA-L injections into the substantia nigra were combined with immunohistochemical localization of tyrosine hydroxylase immunoreactivity to differentiate dopaminergic and non-dopaminergic nigrostriatal projections. Additionally, the distributions of such afferents were compared with the autoradiographic patterns of [^{3}H]-naloxone binding to mu opiate receptors in sections adjacent to those labeled with PHA-L.

3. Other experiments examined the relationship among various biochemical markers that are present in the striatum. These included experiments in which [^{3}H]naloxone binding patterns were compared with the distribution of either somatostatin fiber- or calcium binding protein immunoreactivity, both of which are distributed in a complementary pattern to patches dense in opiate receptors.

Major Findings:

1. A comparison, in adjacent sections through the rat striatum, of [^{3}H]naloxone binding to mu opiate receptors and somatostatin fiber-immunoreactivity showed that these two biochemical markers are distributed in a complementary fashion. These markers were then used as a means of defining the two major neurochemically distinct striatal compartments, with [^{3}H]naloxone binding marking the patch compartment and somatostatin fiber- immunoreactivity marking the matrix compartment. In studies examinining the relationship of connections with these compartments, PHA-L injections into the cortex were used to label corticostriatal inputs and fast blue injections into the substantia nigra were used to retrogradely label striatonigral projection neurons. A comparison of the distribution of such labeled striatal afferent and efferent connections in the same section in which somatostatin fiber-immunoreactivity was used to mark the matrix provided the following results: Somatic sensory, motor and cingulate areas of cortex project selectively to the striatal matrix, whereas the prelimbic cortex, on the medial bank of the frontal pole, projects selectively to the striatal patch compartment. While inputs from individual areas of the somatic sensory and motor areas are somewhat restricted in a topographic manner, the prelimbic inputs are widely distributed and thus interdigitate with inputs from many other cortical areas. Retrograde tracing data show that striatal projection neurons in the matrix project to the substantia nigra pars reticulata whereas striatal projection neurons in the patches project to the substantia nigra pars compacta.

2. The extensive filling of dendrites of striatal projection neuron by transported dye showed further that the labeled portions remained within the boundaries of the compartment in which their cell bodies were located. Together with the specific relationships between cortical areas and striatal compartments these data suggest that striatal input-output system are segregated by the compartmental organization.

3. The somatostatin-immunoreactive neural system provides an intrinsic striatal system linking the two compartments. Somatostatin fibers are distributed principally in the matrix and at least a portion of these arise from neurons that have either cell bodies or dendrites in the patches.

4. The organization of striatal afferents from the substantia nigra and ventral tegmental area were examined in studies combining PHA-L tract tracing, tyrosine hydroxylase TH-immunoreactivity and opiate receptor binding. PHA-L injections into the substantia nigra labeled two distinct types of fiber systems that innervate the striatum. Double labeling of such PHA-L labeled fibers for TH-immunoreactivity show that dopaminergic and non-dopaminergic nigrostriatal afferents are morphologically distinct. Dopaminergic fibers form a lace-like plexus in the striatum and are of fine caliber (0.1-0.2 um) with flattened, thin varicosities (0.2-1.0 um diameter). Non-dopaminergic nigrostriatal fibers are thick (0.5 um diameter) and branch into tree-like arbor bearing large, bulbous boutons (0.15 um diameter).

5. The relationship of nigrostriatal afferents to the opiate receptor rich patches was also examined. Non-dopaminergic nigrostriatal afferents are far less numerous than dopaminergic fibers and were distributed almost exclusively

in the striatal matrix. Dopaminergic afferents originating from the substantia nigra present a variable pattern relative to the patches. Some nigral areas provide inputs that are distributed primarily to patches, some areas project primarily to matrix and others project to both compartments. In contrast, injections into the ventral tegmental area label primarily dopaminergic striatal afferents distributed in the matrix of the ventral striatum. These results show first, that there are distinct dopaminergic and non-dopaminergic nigrostriatal systems and second, that subsets of these projections differentially innervate the striatal compartments. Specifically, the mesolimbic dopaminergic system, originating from the ventral tegmental area, and the dopaminergic nigrostriatal system, appear to differentially innervate the matrix and patch compartments.

6. In a study done in collaboration with K.G. Baimbridge and J.J. Miller at the University of British Columbia, the distributions of brain calcium binding proteins in the basal ganglia were examined. Antisera directed against calcium binding protein (CaBP), purified from human cerebellum, and parvalbumin purified from mouse muscle, were used for immunohistochemical studies in rats and monkeys. CaBP-immunoreactivity is localized in the striatum to projection neurons distributed preferentially in the matrix and labels the projections of these neurons to the substantia nigra. Consistent with tract-tracing studies described above, CaBP-immunoreactive terminals arising from striatal matrix neurons are shown to be distributed amongst GABAergic cells in the substantia nigra pars reticulata and are absent in those nigral areas containing dopaminergic neurons. Thus, CaBP appears to be a select marker of the striatonigral projection system arising from the matrix. Peptides, such as enkephalin, substance P and dynorphin, which are present in both striatal compartments, are also present in nigral afferents distributed to both dopaminergic and non-dopaminergic neurons.

Significance to Biomedical Research and to the Program of the Institute:

In terms of our conceptual understanding of the functional organization of the basal ganglia,the above described results are significant in providing a possible basis for the interactions of the affective and motor functions of the basal ganglia. Previous studies have stressed a regional specialization of the striatum with ventral regions being linked with limbic brain structures and dorsal regions linked with nonlimbic systems subserving regulation of movement. According to these studies the ventral striatal system provided the inputs to dopaminergic neurons in the substantia nigra pars compacta, which, in turn, provided feedback regulation to the dorsal striatum. Such ventral striatal control of the output of the dorsal striatum, which provides inputs through the substantia nigra pars reticulata to brain areas directly involved in controlling movement, had been considered the primary means of interaction between limbic and non-limbic domains in the basal ganglia. The present studies suggest that, in addition to these pathways, there exists "limbic-like" pathways in the dorsal striatum that are connected through the striatal patch compartment.

The significance of the finding (see point #5 above) that there is a difference in the patch/matrix distribution of dopaminergic inputs to the striatum from the ventral tegmental area, on the one hand, and the substantia nigra pars compacta, on the other, may be related to the effects so-called typical and atypical neuro-

leptic drugs have on the physiological responses of these two neuronal cell groups. The potential clinical relevance of this is related to the exacerbation of motor side effects with typical versus atypical neuroleptics in treatments of psychotic behavior. Taken as a whole, the improvements in understanding the normal biochemical, connectional and compartmental organization of the basal ganglia have tremendous potential for future approaches to neuropsychiatric disorders.

Proposed Course of the Project:

The present project, now in its first year, has already led to significant advances in understanding the antomical and chemical organization of the basal ganglia. Follow-up studies, now in progress, are focused on the distribution of CaBP-immunoreactivity in nonhuman primates. Preliminary observations suggest the existence of separate striatonigral systems arising from the matrix and patches and targeting separate neuronal populations in the substantia nigra in both rodents and primates. The possibility that this represents a common feature of mammalian basal ganglia organization will be pursured in future studies. The physiological significance of compartmental organiation in the basal ganglia will be the subject of investigation, beginning within the year, to be conducted by Dr. Melvyn Heyes in collaboration with Gerfen and Wise.

Publications:

Gerfen, C.R.: The neostriatal mosaic: Compartmentalization of corticostriatal input and striatonigral output systems. Nature 311: 461-464, 1984.

Gerfen, C.R.: The neostriatal mosaic: I. Compartmental organization of projections from the striatum to the substantia nigra in the rat. J. Comp. Neurol. 236: 454-476, 1985.

Gerfen, C.R. and Sawchenko, P.E.: A method for anterograde axonal tracing of chemically specified circuits in the central nervous system: Combined PHA-L tract tracing and immunohistochemistry. Brain Research, in press, 1985.

Sawchenko, P.E. and Gerfen, C.R.: Plant lectins and bacterial toxins as tools for tracing neuronal connections. Trends in Neurosci., in press, 1985.

Gerfen, C.R., Baimbridge, K.G., and Miller, J.J.: The neostriatal mosaic: II. Compartmental distribution of calcium binding protein and parvalbumin in the basal ganglia of the rat and primate. Proc. Natl. Acad. Sci. USA in press, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01096-01 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Spatial Organization of the Primate Motor Cortex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Andrew R. Mitz	Staff Fellow	LNP, NIMH
Others:	Steven P. Wise	Research Biologist	LNP, NIMH

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to examine the movement-related organization of the primary motor cortex (MI) and two closely related motor areas, the supplementary motor area (MII) and the premotor area (PM). The model species that has been chosen for study is the rhesus monkey, because the motor areas have been best characterized in this species. In the first part of this project electrical stimulation, including a refinement to the technique of intracortical microstimulation, has been employed to examine the motor topography of MI and MII. A highly detailed view of MII topography has resulted, one that will permit a detailed comparison between its anatomical and physiological organizations.

In the second part of the project, the relationship between single-unit firing patterns and learned movements will be studied to determine whether activity in the three motor areas is best related to the location of a target in space or the direction of movements necessary to acquire the target during a visual tracking task. It is the goal of this part of the project to distinguish each of the motor areas by the relative preponderances of different physiologically-defined cell types in each area.

Objectives:

Movement-related single-unit activity in the primary motor (MI), supplementary motor (MII), and premotor (PM) cortical areas show that these regions are involved in the programming and execution of movements. Anatomical evidence shows that these areas interact closely, and we hypothesize that these three cortical fields act together to generate appropriate motor commands.

Although certain features of the anatomical and physiological organization of these motor areas are known, many questions remain about the detailed topography in each of these areas, their differential connectivity, and their specific contributions to movement programming and execution. Therefore, the objective of this project is to explore the topographical organizations of these three motor areas and correlate their functional organization with aspects of their anatomy, including cytoarchitectonics and connectivity. A further goal is to characterize and distinguish each area's contribution to descending motor commands, in the first instance by probing for differences in the way individual neurons code the spatial organization of movement.

Methods Employed:

1. Microstimulation mapping of MI and MII. Intracortical microstimulation in awake or lightly sedated animals has become the standard tool for examining motor topography in the primary motor cortex, frontal eye fields, and other motor areas. However, this technique has not been successful for exploring the somatotopy of the supplementary motor area (MII). The problem of MII mapping has been overcome in this project by developing a modified intracortical stimulation technique. This technique utilizes platinum-iridium electrodes with about 1000 um^2 of metal exposed at the tip, compared to standard microelectrodes with exposed tips of 100-250 um^2. In addition to the electrode geometry, careful selection of stimulus parameters and the use of biphasic current pulses has contributed to successful MII mapping.

Two rhesus monkeys were implanted with chronic recording chambers over the midline, centered at an anterioposterior position aligned with the posterior limit of the arcuate sulcus. Electrodes were inserted into MII through the dura. Electrical stimuli were delivered periodically along each penetration while two observers recorded the threshold and character of each evoked movement. The stimulus parameters were: 0.2 ms duration for each phase of the biphasic pulses, 330 pulses/second, 31-pulse trains, and 60-65 ua search currents. About 120 penetrations were made into each supplementary motor area and the adjoining part of the primary motor cortex. Penetrations were usually seperated by 1 mm. During some tracks, the animal was traquilized with 1.3 mg/kg ketamine hydrochloride.

2. Connectivity correlated with physiologically-determined topography. Connections among motor areas of the cortex as well as corticofugal projections will be examined by injecting a variety of compatable anterograde and retrograde tracers into areas where electrical stimulation has been used to define the topographical organization. Two techniques will be used for anterograde tracing, autoradiography with tritiated tritiated amino acids and marking of PHA-L

(Phaseolus vulgaris leucoagglutinin), a plant lectin. Two fluorescent dyes, granular blue and diamidino yellow, will be used for retrograde labeling. The post-injection survival time will be 6-12 days, following which the animal will be sacrificed with an overdose of soidium pentobarbital (Somnifer) and perfused with 3% formaldehyde in physiological saline. After brain removal and 12 hours of treatment in 20% sucrose, four one-in-four series of brain sections will be prepared: 1) a Nissl stained series for quantitative cytoarchitectonics using 0.2% thionin, 2) a series mounted on gelatin-coated slides for demonstration of radiolabeled tracers by standard autoradiographic techniques, 3) a series mounted in glycerin for examination of the distribution of fluorescent tracers, and 4) a series incubated in 0.4% Triton X-100 plus 2% normal goat serum in 0.02 M potassium phosphate-buffered saline (pH 7.4), and then incubated at 4°C for 36 hours in guinea pig antisera directed against PHA-L.

3. Chronic single-unit recordings. Movement-related units in the motor areas will be isolated using chronic recording techniques in operantly conditioned monkeys. The current paradigm dissociates direction of movement from target direction. Each animal is being trained to make wrist flexion/extension movements while its forearm is in either a pronated position or a supinated position. When the forearm is pronated, wrist flexion is a downward movement; when the forearm is supinated, wrist flexion is an upward movement. The animal performs these movements in response to a light display signaling the direction and timing of each movement. Units isolated during flexion/extension movements will be tested in both forearm positions. Units will be sampled from the forelimb representations of the primary motor cortex, the supplementary motor area and the premotor cortex. Paradigm events, wrist position, and unit firing times will be recorded in real-time using a Plessy Micro II (PDP 11/23) minicomputer. After each recording session peri-event spike histograms will be generated from the data to compare the event-related activities of individual task-related neurons.

Major Findings:

Controversy concerning the existance and nature of MII topography has stemmed from methodological problems associated with electrical stimulation of MII. Results from previous microstimulation studies of MII have led to the suggestion that this area lacks a motor topography. A new methodological approach, described in the Methods above, has yielded a detailed map of MII motor organization. In this map, orofacial movements, including movements of the pinnae, lips, tongue, and jaw, and conjugate eye movements to the contralateral visual hemifield, were observed most rostrally. Contralateral forelimb movements, including movements of the digits, wrist, forearm, elbow, and shoulder, were evoked from sites immediately caudal to the face area. Hindlimb and tail movement sites were caudal to the forelimb representation, however, preliminary analysis was unable to establish a clear boundary between hindlimb sites of MI and hindlimb sites of MII. The orofacial, forelimb, and hindlimb representations each spanned from the dorsal bank of the cingulate sulcus, to the medial surface of the hemisphere and at least 2 mm onto the hemispheric convexity and medial wall of the hemisphere. The overall rostrocaudal extent of the SMA covered 12-14 mm.

These results have now been confirmed in three hemispheres (of two monkeys) and correlative anatomical studies have confirmed that the supplementary motor cortex, as defined with the modified intracortical stimulation procedures described above, is confined to a part of area 6. (Area 6 is defined here as the agranular cortex in this region lacking giant, layer V pyramidal cells.) It is of particular interest that the MII representation, as defined in this way, includes a representation of eye movements. Historically, eye movements have not been considered a part of motor somatotopy, since these movements were never evoked from the face representation of MI. Thus, the frontal eye fields (FEF) have been presumed to be a seperate MI-like representation for the control of eye movements. However, with the demonstration of eye movements from the face area of MII, the affiliation of the FEF with other motor field should be reconsidered. From its anatomical location rostral to the premotor area and from recent single-unit physiological and cytoarchitectonic studies, there is growing evidence that the FEF might be considered part of the face representation of the premotor area.

Significance to Biomedical Research and to the Program of the Institute:

This project will provide a better understanding of the functional and anatomical organization of cortical motor centers. This study should lead to an improvement of the general understanding of cortical mechanisms and therefore be of value in approaching the problem of cortical dysfunction in humans.

Proposed Course of the Project:

This project is currently in its first year. Intracortical mapping the MII has been completed in the second of three animals, and the preliminary results of this study will be presented at the Society for Neuroscience meeting in October, 1985. The anatomical aspects of the project are in the initial phase as is behavioral training for single-unit recording; the data collection phase of the single-unit recording part of the project will begin before the end of June of this fiscal year.

Publications:

None

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